

# Molecular phylogeny of *Atractus* (Serpentes, Dipsadidae), with emphasis on Ecuadorian species and the description of three new taxa

Alejandro Arteaga<sup>1</sup>, Konrad Mebert<sup>2</sup>, Jorge H. Valencia<sup>3</sup>,  
Diego F. Cisneros-Heredia<sup>4</sup>, Nicolás Peñafiel<sup>5</sup>, Carolina Reyes-Puig<sup>6</sup>,  
José L. Vieira-Fernandes<sup>1</sup>, Juan M. Guayasamin<sup>4,5</sup>

**1** Tropical Herping, Av. 6 de Diciembre N31-50 y Whymper, Torres Santa Fe, Quito, Ecuador **2** Universidade Estadual de Santa Cruz, Departamento de Ciências Biológicas, Rodovia Jorge Amado, Km 16, 45662-900 Salobrinho, Ilhéus, Bahia, Brazil **3** Fundación Herpetológica Gustavo Orcés, Av. Amazonas 3008 y Calle Rumipamba, Casilla Postal 17-033-448, Quito, Ecuador **4** Universidad San Francisco de Quito (USFQ), Colegio de Ciencias Biológicas y Ambientales, Laboratorio de Biología Evolutiva, Laboratorio de Zoología Terrestre, campus Cumbayá, Casilla Postal 17-1200-841, Quito, Ecuador **5** Centro de Investigación de la Biodiversidad y Cambio Climático (BioCamb), Facultad de Ciencias de Medio Ambiente, Ingeniería en Biodiversidad y Recursos Genéticos, Universidad Tecnológica Indoamérica, Av. Machala y Sabanilla, Quito, Ecuador **6** Museo Ecuatoriano de Ciencias Naturales, Instituto Nacional de Biodiversidad, División de Herpetología, calle Rumipamba 341 y Av. de los Shyris, Casilla 17-07-8976, Quito, Ecuador

Corresponding author: Alejandro Arteaga ([alejandro@tropicalherping.com](mailto:alejandro@tropicalherping.com))

---

Academic editor: R. Jadin | Received 17 November 2016 | Accepted 8 March 2017 | Published 15 March 2017

---

<http://zoobank.org/7CBF7FB1-EFEA-4DC1-8F64-5BF862694AA0>

---

**Citation:** Arteaga A, Mebert K, Valencia JH, Cisneros-Heredia DF, Peñafiel N, Reyes-Puig C, Vieira-Fernandes JL, Guayasamin JM (2017) Molecular phylogeny of *Atractus* (Serpentes, Dipsadidae), with emphasis on Ecuadorian species and the description of three new taxa. ZooKeys 661: 91–123. <https://doi.org/10.3897/zookeys.661.11224>

---

## Abstract

We present a molecular phylogeny of snake genus *Atractus*, with an improved taxon sampling that includes 30 of the 140 species currently recognized. The phylogenetic tree supports the existence of at least three new species in the Pacific lowlands and adjacent Andean slopes of the Ecuadorian Andes, which we describe here. A unique combination of molecular, meristic and color pattern characters support the validity of the new species. With the newly acquired data, we propose and define the *A. iridescens* species

group, as well as redefine the *A. roulei* species group. The species *A. iridescens* is reported for the first time in Ecuador, whereas *A. bocourti* and *A. medusa* are removed from the herpetofauna of this country. We provide the first photographic vouchers of live specimens for *A. multicinctus*, *A. paucidens* and *A. touzeti*, along with photographs of 19 other Ecuadorian *Atractus* species. The current status of *A. occidentalis* and *A. paucidens* is maintained based on the discovery of new material referable to these species. With these changes, the species number reported in Ecuador increases to 27, a number that is likely to increase as material not examined in this work becomes available and included in systematic studies.

## Keywords

Pacific lowlands, biodiversity, Ecuador, groundsnakes, *Atractus*, phylogeny, new species

## Introduction

With 140 species, *Atractus* is the most speciose snake genus in the world, with 33 new species described only during the last ten years (Uetz et al. 2016). Most of these new species have been described using a combination of meristic and morphometric characters (Passos et al. 2009a, 2016, Passos and Lynch 2010, Schargel et al. 2013, Salazar-Valenzuela et al. 2014). However, with the exception of the preliminary phylogeny presented in De Oliveira and Hernández-Ruz (2016), no studies have involved a phylogenetic approach to test species arrangements and boundaries.

One recent work by Passos et al. (2009a) evaluated the taxonomic status of *Atractus* species from the Pacific lowland of Colombia and Ecuador, using a combination of meristic, morphometric, color pattern, and hemipenial characters. These authors described three new species and provided a comprehensive review of all *Atractus* known to occur in the region. However, when referring to this work to compare previously unexamined material from Ecuador, it became clear to us that several Ecuadorian specimens of Pacific lowland *Atractus* could not be assigned to any taxa currently recognized to occur in the country. Some specimens identified as *A. medusa* (Passos et al. 2009a) matched the coloration of the first specimen reported in Ecuador by Cisneros-Heredia and Romero (2015), but they did not match the coloration of the holotype (Passos et al. 2009a). Other specimens were closer in coloration and lepidosis to *A. iridescens* (Peracca, 1860) from Colombia, and others resembled both *A. microrhynchus* (Cope, 1868) and *A. occidentalis* (Savage, 1955). To further complicate matters, the taxonomic validity of *A. occidentalis* and *A. paucidens* (Despax, 1910) was not recognized in Arteaga et al. (2013), owing to their close morphological resemblance to *A. dunni* (Savage, 1955) and *A. modestus* (Boulenger, 1894), respectively.

To resolve these pending issues and to shed light on potentially unclear species boundaries, we report on new material of *Atractus* from Ecuador, review current knowledge on the species occurring in the Pacific lowlands and adjacent Andean slopes, present a new molecular phylogeny, including most Ecuadorian species, and describe three new species of *Atractus*.

## Materials and methods

### Ethics statement

This study was carried out in strict accordance with the guidelines for use of live amphibians and reptiles in field research compiled by the American Society of Ichthyologists and Herpetologists (ASIH), The Herpetologists' League (HL) and the Society for the Study of Amphibians and Reptiles (SSAR). All procedures with animals (see below) were approved by the Centro de Investigación de la Biodiversidad y Cambio Climático (BioCamb) of the Universidad Tecnológica Indoamérica. They also were reviewed by the Ministerio de Ambiente del Ecuador (MAE) and specifically approved as part of obtaining the following field permits for research and collection: MAE-DNB-CM-2015-0017, granted to Universidad Tecnológica Indoamérica; and permit N°012-IC-FAN-DPEO-MAE, granted to the Museo Ecuatoriano de Ciencias Naturales. Specimens were euthanized with 20% benzocaine, fixed in 10% formalin or 70% ethanol, and stored in 70% ethanol. Museum vouchers were deposited at the Museo de Zoología of the Universidad Tecnológica Indoamérica (MZUTI).

### Sampling

Tissue samples from 39 individuals representing 22 species (including three new species described here) were obtained throughout Ecuador. The majority of individuals were located by space-constrained visual examination of ground-level substrates (Campbell and Christman 1982). The remaining individuals were detected by turning over logs, rocks and other surface objects. All specimens included in the genetic analyses were morphologically identified according to Savage (1955, 1960), Cisneros-Heredia (2005), Passos et al. (2009a), Arteaga et al. (2013), Schargel et al. (2013) and Salazar-Valenzuela et al. (2014). We generated sequence data for samples marked with an asterisk under Appendix I, which includes museum vouchers at the Museo de Zoología de la Universidad Tecnológica Indoamérica (MZUTI), the División de Herpetología del Museo Ecuatoriano de Ciencias Naturales (DHMECN) and the Fundación Herpetológica Gustavo Orcés (FHGO).

### Laboratory techniques

Genomic DNA was extracted from 96% ethanol-preserved tissue samples (liver, muscle tissue or scales) using a modified salt precipitation method based on the Puregene DNA purification kit (Gentra Systems). We amplified the 16S gene using the primers 16Sar-L and 16Sbr-H-R from Palumbi et al. (1991). Additionally, the Cytb gene was obtained with the primers L14910 and H16064 developed by Burbrink et al. (2000), whereas the gene coding for the subunit 4 of the NADH dehydrogenase was ampli-

fied with the primers ND4 and Leu developed by Arévalo et al. (1994). PCR reactions contained 2 mM (Cytb and ND4) or 3 mM (16S)  $\text{MgCl}_2$ , 200  $\mu\text{M}$  dNTP mix, 0.2  $\mu\text{M}$  (16S and Cytb) or 0.8  $\mu\text{M}$  (ND4) of each primer and 1.25 U (16S and Cytb) or 0.625 U (ND4) Taq DNA Polymerase Recombinant (Thermo Fisher Scientific) in a 25  $\mu\text{L}$  total volume. The nucleotide sequences of the primers and the PCR conditions applied to each primer pair are detailed in Appendix II. PCR products were cleaned with Exonuclease I and Alkaline Phosphatase (Illustra ExoProStar by GE Healthcare) before they were sent to Macrogen Inc (Korea) for sequencing. All PCR products were sequenced in both forward and reverse directions with the same primers that were used for amplification. The edited sequences were deposited in GenBank (Appendix I).

### DNA sequence analyses

A total of 126 mtDNA sequences were used to build a mitochondrial phylogenetic tree of the genus *Atractus*. 69 were generated during this work and 57 (all available sequences for the sampled gene fragments) were downloaded from GenBank. A mitochondrial marker dataset, though less powerful to study higher-level phylogenetic relationships, was chosen because it is the most effective to successfully resolve species-level phylogenies (Patwardhan 2014). Recently published works looking to resolve intrageneric relationships within Neotropical dipsadines have done so using phylogenies that are largely based on mitochondrial data (Krysko et al. 2015, Pyron et al. 2016). Specifically, we use the gene Cytochrome-b because it is reported as the most powerful in recovering phylogenetic relationships among closely related taxa (Patwardhan 2014), which is the case for the species of *Atractus* studied here. The mitochondrial genes 16S and ND4 were used to be able to compare with *Atractus* sequences available in GenBank. Novel sequences were edited and assembled using the program Geneious Pro™ 5.4.7 (Drummond et al. 2010) and aligned with those downloaded from Genbank (Appendix I) using MAFFT v.7 (Katoh and Standley 2013) under the default parameters in Geneious Pro™ 5.4.7. Genes were combined into a single matrix with seven partitions, one per non-coding gene and three per protein coding gene corresponding to each codon position. The best partition strategies along with the best-fit models of evolution were obtained in PartitionFinder 1.1.1 (Lanfear et al. 2012) and jModeltest (Darriba et al. 2012) under the Bayesian information criterion. Phylogenetic relationships were assessed under a Bayesian approach in MrBayes 3.2.0 (Ronquist and Huelsenbeck 2013). Four independent analyses were performed to reduce the chance of converging on a local optimum. Each analysis consisted of 6.7 million generations and four Markov chains with default heating settings. GenBank accession numbers are listed in Appendix I. Trees were sampled every 1,000 generations, resulting in 5,000 saved trees per analysis after 25% of those were arbitrarily discarded as “burn-in.” Stationarity was confirmed by plotting the  $-\ln L$  per generation in the program Tracer 1.2 (Rambaut and Drummond 2003). Genetic distances between *A. esepe* and its closest morphological relatives were calculated using the uncorrected distance matrix in PAUP 4.0 (Swofford 2002).

## Morphological data

Our terminology for *Atractus* cephalic shields follows Savage (1960), diagnoses and descriptions generally follow Passos et al. (2009a), and ventral and subcaudal counts follow Dowling (1951). We examined comparative alcohol-preserved specimens from the herpetology collections at the MZUTI, DHMECN, Fundación Herpetológica Gustavo Orcés (FHGO), Museum d'Histoire Naturelle de la Ville de Genève (MHNG), Museo de Historia Natural de la Escuela Politécnica Nacional (EPN), Museo de Zoología de la Pontificia Universidad Católica del Ecuador (QCAZ), National Museum of Natural History (USNM), Muséum National d'Histoire Naturelle (MNHN) and Museo de Zoología de la Universidad San Francisco de Quito (ZSFQ). (Table 1). Morphological measurements were taken with measuring tapes to the nearest 1 mm. When providing the standard deviation, we use the  $\pm$  symbol. Sex was determined by noting the presence or absence of hemipenes through a subcaudal incision at the base of the tail.

## Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/". The LSID for this publication is: urn:lsid:zoobank.org:pub:7CBF7FB1-EFEA-4DC1-8F64-5BF862694AA0. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS.

## Results

### Molecular phylogeny

The overall topology and support (Fig. 1) is similar to that of Pyron et al. (2015). We consider strong support to be posterior probability values  $>95\%$ , following Felsenstein (2004). Overall, there is low support for many backbone nodes. Strong support was found for the clade colored in yellow under Fig. 1.

The resulting hypotheses of species relationships for our mitochondrial phylogenetic tree supports Savage's (1960) assumption suggesting independent evolution of the 15 dorsal scale row lineage within *Atractus*, since species with this number of dorsal scale rows, like *A. elaps*, *A. roulei* and *A. duboisi*, belong to different lineages. However, the tree does show that *A. carrioni* (Parker 1930), *A. lehmanni* (Boettger 1898),



**Table 1.** Locality data for specimens examined in this study. Coordinates represent georeferencing attempts from gazetteers under standard guidelines, though some variation from the exact collecting locality will be present. Similarly, elevations are taken from Google Earth, and may not exactly match the elevations as originally reported.

Species	Voucher	Locality	Latitude	Longitude	Elev.
<i>A. carrioni</i>	DHMECN 4697	Loja, Utuana	-4.36642	-79.72483	2517
<i>A. carrioni</i>	DHMECN 76	Esmeraldas, Copa Quinde (in error)	0.06181	-78.72641	1688
<i>A. carrioni</i>	DHMECN 7668	Loja, Utuana	-4.36642	-79.72483	2517
<i>A. carrioni</i>	MZUTI 4194	Loja, Utuana	-4.36642	-79.72483	2517
<i>A. carrioni</i>	MZUTI 4195	Loja, Utuana	-4.36642	-79.72483	2517
<i>A. duboisi</i>	MHNG 2457.093	Napo, Chiriboga (in error)	-	-	-
<i>A. duboisi</i>	MNHN 0.6147	Ecuador	-	-	-
<i>A. duboisi</i>	MZUTI 3640	Napo, Yanayacu	-0.60071	-77.88927	1924
<i>A. duboisi</i>	MZUTI 62	Napo, Yanayacu	-0.59939	-77.89050	2064
<i>A. dunni</i>	DHMECN 12769	Carchi, Gualpi	0.86439	-78.22435	2104
<i>A. dunni</i>	DHMECN 2215	Pichincha, Río Cambugán	0.17697	-78.50779	1828
<i>A. dunni</i>	DHMECN 3527	Imbabura, Junín	0.27009	-78.64975	1688
<i>A. dunni</i>	DHMECN 3900	Pichincha, Tambo Quinde	0.00967	-78.66906	1870
<i>A. dunni</i>	DHMECN 4159	Pichincha, Pahuma	0.02757	-78.63208	1914
<i>A. dunni</i>	EPN 3127	Santo Domingo, Chiriboga	-0.22841	-78.76725	1813
<i>A. dunni</i>	EPN 3128	Santo Domingo, Chiriboga	-0.22841	-78.76725	1813
<i>A. dunni</i>	FHGO 375	Santo Domingo, La Favorita	-0.22833	-78.76503	1810
<i>A. dunni</i>	FHGO 376	Santo Domingo, La Favorita	-0.22833	-78.76503	1810
<i>A. dunni</i>	FHGO 379	Santo Domingo, La Favorita	-0.22833	-78.76503	1810
<i>A. dunni</i>	FHGO 91	Santo Domingo, La Favorita	-0.22833	-78.76503	1810
<i>A. dunni</i>	MHNG 2441.043	Cotopaxi, Cutzualo	-0.54497	-78.91891	1952
<i>A. dunni</i>	MHNG 2457.091	Santo Domingo, La Favorita	-0.22841	-78.76725	1813
<i>A. dunni</i>	MHNG 2464.03	Cotopaxi, Otonga	-0.41549	-79.00480	2095
<i>A. dunni</i>	MZUTI 2189	Pichincha, Tandayapa–Bellavista	-0.00843	-78.67619	1919
<i>A. dunni</i>	MZUTI 3031	Pichincha, Tandayapa Lodge	0.00268	-78.68131	1757
<i>A. dunni</i>	MZUTI 4097	Imbabura, Santa Rosa de Intag	0.37616	-78.46054	2077
<i>A. dunni</i>	MZUTI 4098	Imbabura, Santa Rosa de Intag	0.37616	-78.46054	2077
<i>A. dunni</i>	MZUTI 4099	Imbabura, Santa Rosa de Intag	0.37616	-78.46054	2077
<i>A. dunni</i>	MZUTI 4100	Imbabura, Below of Siempre Verde	0.37782	-78.46901	1974
<i>A. dunni</i>	MZUTI 4318	Imbabura, Toisán	0.53297	-78.52924	2286
<i>A. dunni</i>	MZUTI 4319	Imbabura, Toisán	0.53297	-78.52924	2286
<i>A. dunni</i>	ZSFQ 1513	Santo Domingo, Guajalito	-0.22875	-78.82248	1801
<i>A. ecuadorensis</i>	DHMECN 5101	Tungurahua, Río Verde	-1.40344	-78.30099	1507
<i>A. elaps</i>	DHMECN 10179	Morona Santiago, Tundayme	-3.57244	-78.46982	790
<i>A. gaigeae</i>	MHNG 2397.044	Morona Santiago, Macas	-2.31670	-78.11670	972
<i>A. gigas</i>	MHNG 2250.035	Santo Domingo, Chiriboga	-0.22841	-78.76725	1813
<i>A. gigas</i>	MHNG 2441.02	Cotopaxi, Otonga	-0.41549	-79.00480	2095
<i>A. gigas</i>	MZUTI 3286	Pichincha, Las Gralarias	-0.00807	-78.73238	1985
<i>A. iridescens</i>	DHMECN 2932	Esmeraldas, Canande	0.52993	-79.03541	594
<i>A. iridescens</i>	DHMECN 5663	Esmeraldas, Tundaloma	1.18236	-78.75250	74
<i>A. iridescens</i>	DHMECN 9633	Esmeraldas, Canande	0.52993	-79.03541	594
<i>A. iridescens</i>	EPN 13920	Carchi, Río Blanco	1.18993	-78.50413	223

Species	Voucher	Locality	Latitude	Longitude	Elev.
<i>A. iridescens</i>	FHGO 10443	Esmeraldas, Tsejpi	0.79930	-78.84527	152
<i>A. iridescens</i>	MZUTI 3548	Esmeraldas, Tundaloma	1.18166	-78.74945	74
<i>A. iridescens</i>	MZUTI 3680	Esmeraldas, Tundaloma	1.18166	-78.74945	74
<i>A. iridescens</i>	MZUTI 4178	Pichincha, Puerto Quito	0.11667	-79.26661	143
<i>A. iridescens</i>	MZUTI 4697	Esmeraldas, Canande	0.52993	-79.03541	594
<i>A. iridescens</i>	ZSFQ 191.101109	Esmeraldas, Tundaloma	1.18166	-78.74945	74
<i>A. lehmanni</i>	DHMECN 7644	Azuay, Reserva Yunguilla	-3.22684	-79.27520	1748
<i>A. lehmanni</i>	DHMECN 7645	Azuay, Reserva Yunguilla	-3.22684	-79.27520	1748
<i>A. major</i>	ANF 1545	Orellana, Estación Científica Yasuní	-0.67781	-76.39819	246
<i>A. major</i>	DHMECN 8343	Sucumbíos, Bloque 27	0.32273	-76.19369	272
<i>A. major</i>	MNHN 0.6149	Ecuador	-	-	-
<i>A. major</i>	MZUTI 4973	Zamora Chinchipe, Maycu	-4.38030	-78.74584	981
<i>A. microrhynchus</i>	DHMECN 2536	El Oro, Buenaventura	-3.65467	-79.76794	524
<i>A. microrhynchus</i>	DHMECN 2586	El Oro, Buenaventura	-3.65467	-79.76794	524
<i>A. microrhynchus</i>	FHGO 897	El Oro, Zambo Tambo	-3.67861	-79.68001	1014
<i>A. microrhynchus</i>	MHNG 2307.017	El Oro, El Progreso	-3.26998	-79.73452	176
<i>A. microrhynchus</i>	MHNG 2397.019	El Oro, El Progreso	-3.26998	-79.73452	176
<i>A. microrhynchus</i>	MHNG 2397.02	El Oro, El Progreso	-3.26998	-79.73452	176
<i>A. microrhynchus</i>	MHNG 2397.021	El Oro, El Progreso	-3.26998	-79.73452	176
<i>A. microrhynchus</i>	MHNG 2459.052	El Oro, El Progreso	-3.26998	-79.73452	176
<i>A. microrhynchus</i>	MZUTI 4122	Manabí, Jama Coaque	-0.11556	-80.12472	299
<i>A. microrhynchus</i>	MZUTI 5109	Los Ríos, Río Palenque	-0.59273	-79.36369	163
<i>A. microrhynchus</i>	QCAZ 1219	Loja, Olmedo	-3.94994	-79.66667	1545
<i>A. microrhynchus</i>	USNM 285473	Los Ríos, Río Palenque	-0.58333	-79.36667	173
<i>A. microrhynchus</i>	USNM 285474	Los Ríos, Río Palenque	-0.58333	-79.36667	173
<i>A. modestus</i>	DHMECN 3859	El Oro, Piñas	-3.68041	-79.68253	1019
<i>A. modestus</i>	EPN 13916	Carchi, Chical	0.90327	-78.16201	1437
<i>A. modestus</i>	FHGO 2936	Pichincha, Maquipucuna	0.11757	-78.67446	1490
<i>A. modestus</i>	FHGO 44	Pichincha, Maquipucuna	0.11757	-78.67446	1490
<i>A. modestus</i>	MHNG 2397.041	Cotopaxi, Las Pampas	-0.44036	-78.96663	1590
<i>A. modestus</i>	MZUTI 4760	Pichincha, Guala	0.08536	-78.74092	1557
<i>A. multicinctus</i>	MZUTI 5106	Esmeraldas, Canandé	0.52581	-79.2088	310
<i>A. occidentalis</i>	EPN 13077	Pichincha, Mindo	-0.04872	-78.77520	1277
<i>A. occidentalis</i>	FHGO 385	Santo Domingo, La Favorita	-0.22833	-78.76503	1810
<i>A. occidentalis</i>	MHNG 2252.079	Cotopaxi, Las Pampas	-0.44036	-78.96663	1590
<i>A. occidentalis</i>	MHNG 2307.068	Pichincha, Tandapi	-0.41522	-78.79728	1455
<i>A. occidentalis</i>	MHNG 2397.028	Cotopaxi, Las Pampas	-0.44036	-78.96663	1590
<i>A. occidentalis</i>	MHNG 2411.085	Pichincha, Tandapi	-0.41522	-78.79728	1455
<i>A. occidentalis</i>	MHNG 2411.086	Pichincha, Tandapi	-0.41522	-78.79728	1455
<i>A. occidentalis</i>	MHNG 2441.044	Pichincha, Nanegalito	0.06181	-78.72641	1688
<i>A. occidentalis</i>	MZUTI 1385	Pichincha, Yellow House	-0.04492	-78.75843	1504
<i>A. occidentalis</i>	MZUTI 2649	Pichincha, Yellow House	-0.05199	-78.76923	1325
<i>A. occidentalis</i>	MZUTI 2650	Pichincha, Yellow House	-0.04371	-78.75351	1520
<i>A. occidentalis</i>	MZUTI 3323	Pichincha, Las Gralarias	-0.00615	-78.73381	1985
<i>A. paucidens</i>	DHMECN 11980	Pichincha, Pedro Vicente Maldonado	0.05361	-78.92109	938
<i>A. paucidens</i>	DHMECN 3975	Santa Elena, Comuna Loma Alta	-1.83442	-80.70291	72

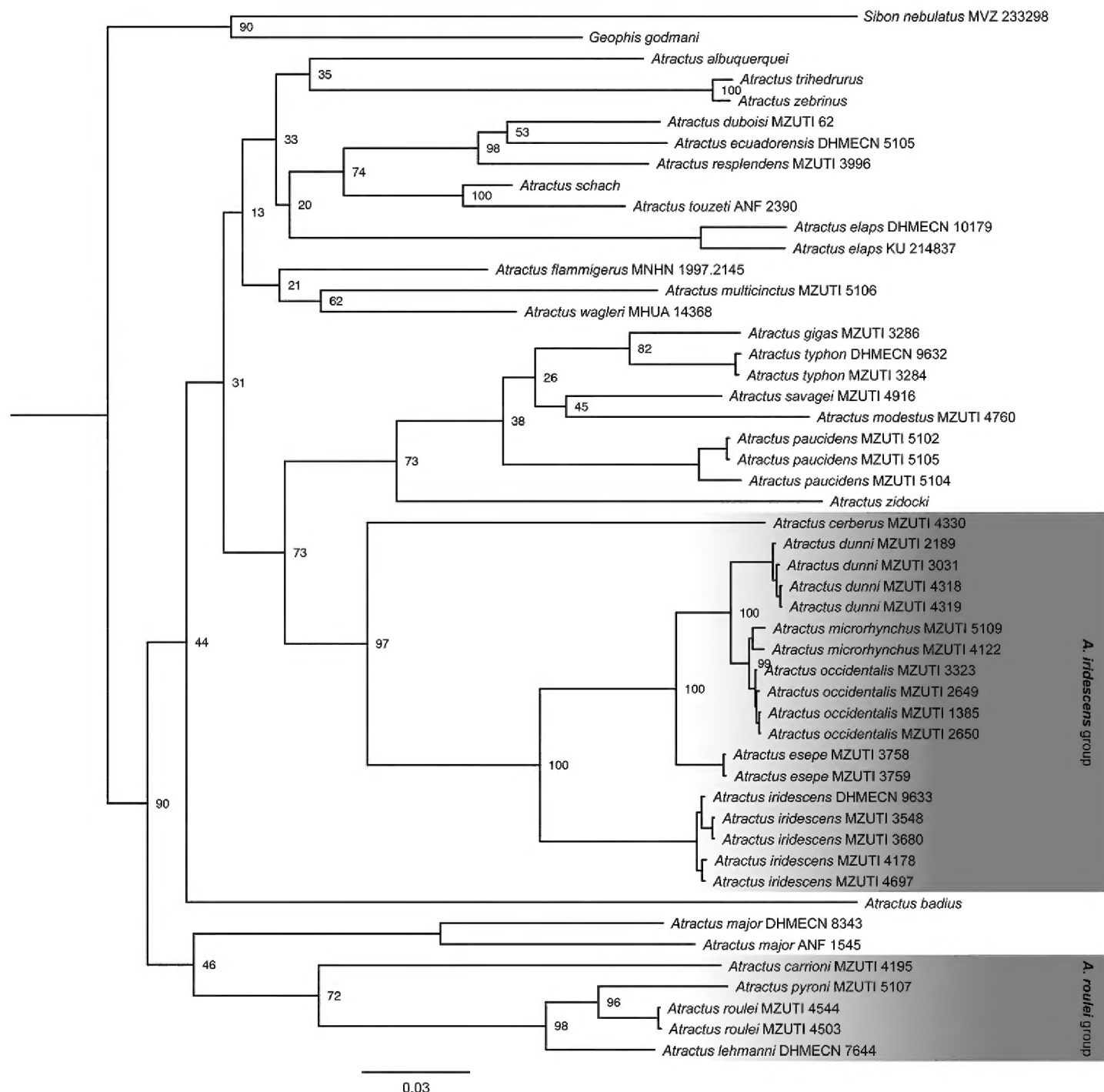
Species	Voucher	Locality	Latitude	Longitude	Elev.
<i>A. paucidens</i>	EPN 8729	Santo Domingo, Finca La Esperanza	-0.27160	-79.10568	616
<i>A. paucidens</i>	EPN 8730	Santo Domingo, Finca La Esperanza	-0.27160	-79.10568	616
<i>A. paucidens</i>	EPN 8731	Santo Domingo, Finca La Esperanza	-0.27160	-79.10568	616
<i>A. paucidens</i>	EPN 8732	Santo Domingo, Finca La Esperanza	-0.27160	-79.10568	616
<i>A. paucidens</i>	MHNG 2309.065	Pichincha, Puerto Quito	0.11667	-79.26661	143
<i>A. paucidens</i>	MNHN 1906.245	Santo Domingo, Santo Domingo	-0.25351	-79.17297	554
<i>A. paucidens</i>	MZUTI 5102	Pichincha, Río Cinto	-0.09070	-78.80299	1409
<i>A. paucidens</i>	MZUTI 5104	El Oro, Buenaventura	-3.65467	-79.76794	524
<i>A. paucidens</i>	MZUTI 5105	Pichincha, Río Cinto	-0.09070	-78.80299	1409
<i>A. resplendens</i>	MZUTI 3996	Tungurahua, Puntzan	-1.41359	-78.40951	1962
<i>A. roulei</i>	MZUTI 4503	Chimborazo, Vicinity of Tixán	-2.16174	-78.81227	2892
<i>A. roulei</i>	MZUTI 4544	Chimborazo, Vicinity of Tixán	-2.16174	-78.81227	2892
<i>A. roulei</i>	QCAZ 6256	Azuay, Hierba Mala	-2.76439	-79.43816	3029
<i>A. roulei</i>	QCAZ 7887	El Oro, Guanazán	-3.44139	-79.49417	2596
<i>A. roulei</i>	QCAZ 7902	El Oro, Guanazán	-3.44139	-79.49417	2596
<i>A. roulei</i>	QCAZ 9643	El Oro, Guanazán	-3.44139	-79.49417	2596
<i>A. roulei</i>	QCAZ 9652	El Oro, Guanazán	-3.44139	-79.49417	2596
<i>A. savagei</i>	DHMECN 3800	Carchi, Río la Plata	0.82381	-78.04584	2256
<i>A. savagei</i>	MZUTI 4916	Carchi, Chilma Bajo	0.86495	-78.04978	2058
<i>A. snethlageae</i>	MNHN 1906.244	Morona Santiago, Gualaquiza	-3.39914	-78.57859	835
<i>A. snethlageae</i>	MNHN 1994.1171	Morona Santiago, Gualaquiza	-3.39914	-78.57859	835
<i>A. touzeti</i>	ANF 2390	Pastaza, Tzarentza	-1.35696	-78.05814	1355
<i>A. trilineatus</i>	MNHN 1898.313	Imbabura, Paramba (in error)	0.81671	-78.35002	698
<i>A. trilineatus</i>	MNHN 1898.314	Imbabura, Paramba (in error)	0.81671	-78.35002	698
<i>A. typhon</i>	DHMECN 9632	Esmeraldas, Canandé	0.52993	-79.03541	594
<i>A. typhon</i>	FHGO 10438	Esmeraldas, Gualpi	0.78173	-79.15993	63
<i>A. typhon</i>	FHGO 10439	Esmeraldas, Gualpi	0.78173	-79.15993	63
<i>A. typhon</i>	MZUTI 3284	Esmeraldas, Itapoa	0.51307	-79.13400	321

*A. roulei* (Despax, 1910) and *A. pyroni* sp. n., species with 15 scale rows, form a monophyletic group that includes two more species than was suggested by Passos et al. (2013) when naming the *A. roulei* species group (Fig. 1).

*Atractus gigas* (Myers and Schargel, 2006), *A. modestus*, *A. paucidens*, *A. savagei* (Salaazar-Valenzuela et al. 2014), *A. typhon* (Passos et al., 2009a) and *A. zidoki* (Gasc and Rodrigues, 1979) form a poorly supported clade that does not include *A. microrhynchus* and *A. iridescens*, as was suggested by Passos et al. (2009a) when naming the *A. paucidens* species group (Fig. 1). Six species, *Atractus cerberus* sp. n., *A. dunni*, *A. esepe* sp. n., *A. iridescens*, *A. microrhynchus*, and *A. occidentalis*, form a strongly supported clade sister to the *A. paucidens* species group. Here, we name this lineage as the *A. iridescens* species group (Fig. 1).

*Atractus occidentalis* forms a strongly supported distinct lineage, sister to *A. microrhynchus*. Together, these two species are sister to *A. dunni*. *Atractus typhon* is shown to be the strongly supported sister lineage of *A. gigas*, as is the case for a relationship between *A. roulei* and *A. pyroni* sp. n.





**Figure 1.** Bayesian consensus phylogeny depicting relationships within colubrid snakes of the genus *Atractus*, summarized from 5 million post-burnin generations in MrBayes 3.2.0. The topology was derived from analysis of 2,564 bp of mitochondrial DNA (gene fragments 16S, Cytb and ND4). Numbers next to branches correspond to posterior probability values. PP values on intraspecific branches are not shown for clarity. Voucher numbers for sequences are indicated for each terminal when available.

### New taxa and systematic arrangements derived from the analyses

We seek here to only name or redelimit *Atractus* species groups that are supported in our molecular phylogeny and share features of their coloration pattern and lepidosis. The first such groups is the clade comprising *A. cerberus* sp. n., *A. dunni*, *A. esepe* sp. n., *A. iridescens*, *A. microhynchus* and *A. occidentalis*. The other is the one comprising *A. carrioni*, *A. lehmanni*, *A. pyroni* sp. n. and *A. roulei*.

*Atractus iridescens* species group

**Diagnosis.** 200–360 mm SVL *Atractus* with brown dorsal ground color bearing a pattern of dots or stripes (Fig. 2), generally 17/17/17 smooth dorsals, and 125–163 ventrals (Table 2).

**Content.** *Atractus cerberus* sp. n., *A. dunni*, *A. echidna*, *A. esepe* sp. n., *A. iridescens*, *A. microrhynchus* and *A. occidentalis*.

**Distribution.** Pacific lowlands and western Andean slopes in Ecuador and Colombia (Fig. 3).

**Comment.** Passos et al. (2009a) included *Atractus echidna*, *A. iridescens* and *A. microrhynchus* in the phenetic *A. paucidens* species group. Later, Passos et al. (2012) placed *A. microrhynchus* in the *A. multicinctus* group based on hemipenial characters. Unlike *A. paucidens* or *A. multicinctus* (Jan, 1865), however, the former three species have a brownish color pattern (Fig. 2) and also a lower number of ventral scales (Appendix III). These differences, together with the phylogenetic placement of *A. iridescens* and *A. microrhynchus* support the allocation of these species in the newly formed *A. iridescens* group.

*Atractus roulei* species group

**Diagnosis.** 300–450 mm SVL *Atractus* with olive to grayish brown dorsal ground color lacking dots and stripes, 15/15/15 smooth dorsals (occasionally 17/17/17), generally 6 supralabials (sometimes 5), and 135–161 ventrals (Table 3).

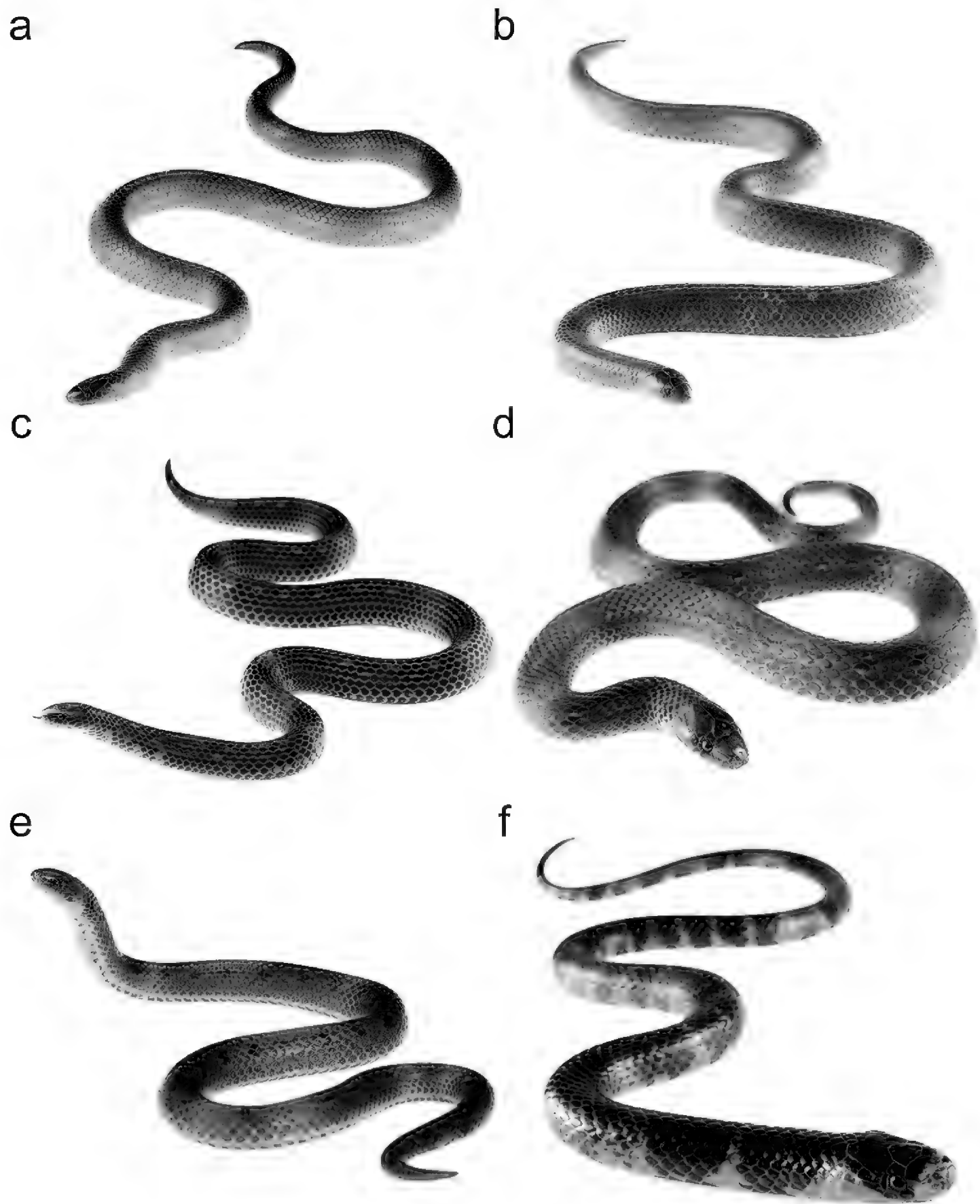
**Content.** *Atractus carrioni*, *A. lehmanni*, *A. pyroni* sp. n. and *A. roulei* (Fig. 1).

**Distribution.** Western slopes of the Andes and inter-Andean valleys in central and southern Ecuador (Fig. 4).

**Comment.** Passos et al. (2013) created the *Atractus roulei* species group to accommodate *A. roulei* and its closest morphological relative *A. carrioni*, based mainly on

**Table 2.** Morphometric data for members of the *Atractus iridescens* species group. Codes are: V=ventrals; SC=subcaudals; D=dorsal scale rows at midbody; PO=postoculars; SL=supralabials; IL=infralabials; MT=maxillary teeth. Data is derived from Appendix III and from the literature.

Species	V		SC		D	PO	SL	IL	MT
	Males	Females	Males	Females					
<i>A. cerberus</i>	152–157	–	25–26	–	17	2	7	7	7
<i>A. dunni</i>	125–136	138–150	26–39	19–26	17	2	6–7	6–8	5–7
<i>A. echidna</i>	127	–	36	–	15	2	7	7	6
<i>A. esepe</i>	149	156	41	30	17	2	7	7	5
<i>A. iridescens</i>	127–150	135–144	33–42	25–37	17	2	6–7	6–7	5–6
<i>A. microrhynchus</i>	133–150	144–163	32–40	24–29	17	1–2	7	6–7	5–7
<i>A. occidentalis</i>	129–141	128–149	33–39	20–37	17	2	6–7	6–7	5–7



**Figure 2.** Photographs of some Ecuadorian species of *Atractus* in life: *A. carrioni* MZUTI 4194 (a), MZUTI 4195 (b), *A. duboisi* MZUTI 3640 (c), *A. dunni* MZUTI 4318 (d), *A. dunni* MZUTI 2189 (e), *A. elaps* AMARU SN (f), *A. gigas* MZUTI 3286 (g), *A. iridescens* MZUTI 3680 (h), *A. iridescens* QCAZ 8072 (i), *A. iridescens* MZUTI 4697 (j), *A. iridescens* MZUTI 3548 (k), *A. major* MZUTI 4973 (l), *A. microrhynchus* MZUTI 5109 (m), *A. modestus* (n), *A. multicinctus* MZUTI 5106 (o), *A. occidentalis* MZUTI 1385 (p), *A. occidentalis* MZUTI 3323 (q), *A. paucidens* MZUTI 5102 (r), *A. resplendens* MZUTI 3996 (s), *A. roulei* MZUTI 4503 (t), *A. savagei* MZUTI 4916 (u), *A. snethlageae* (v), *A. touzeti* ANF 2390 (w), and *A. typhon* MZUTI 5110.

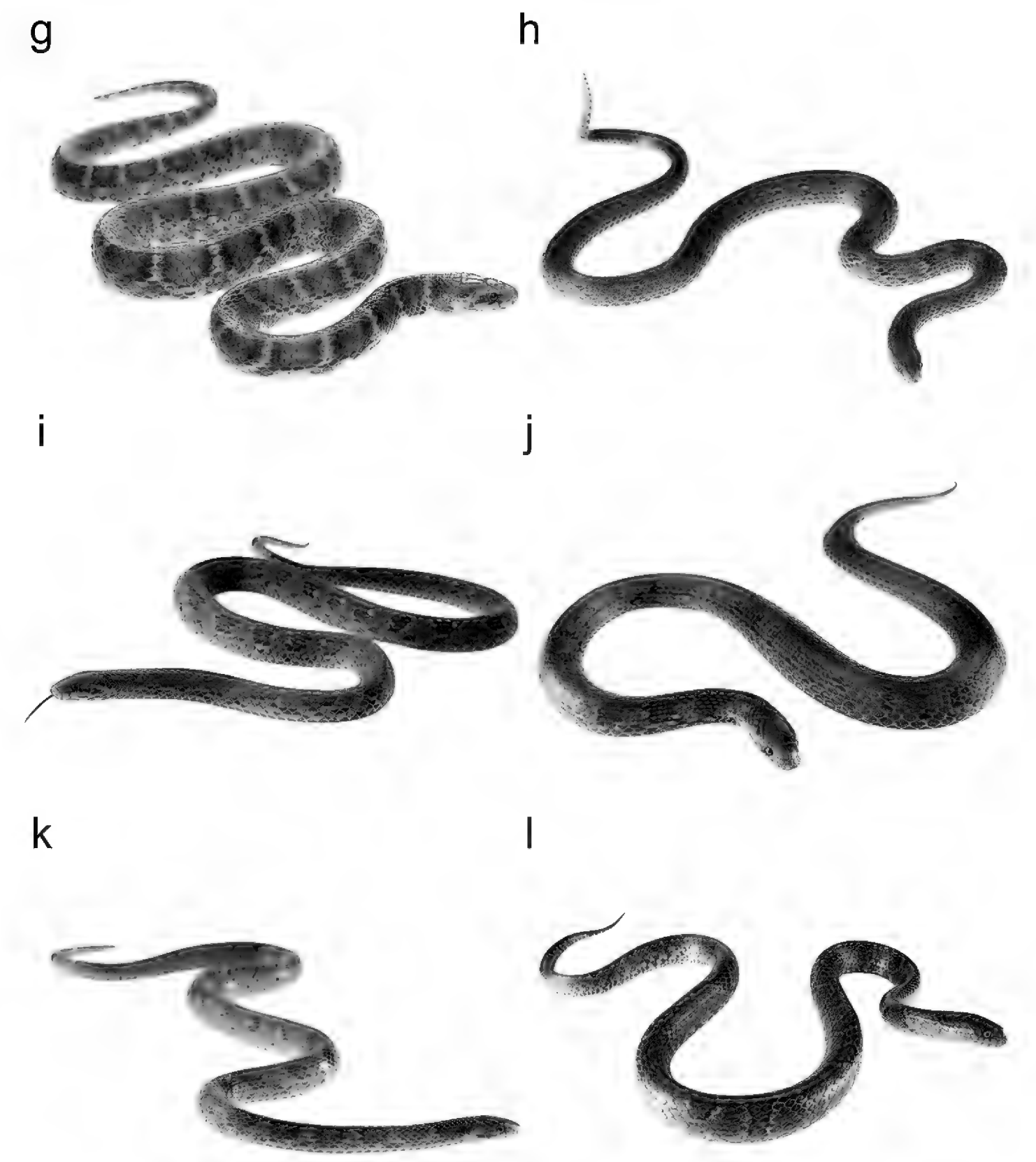
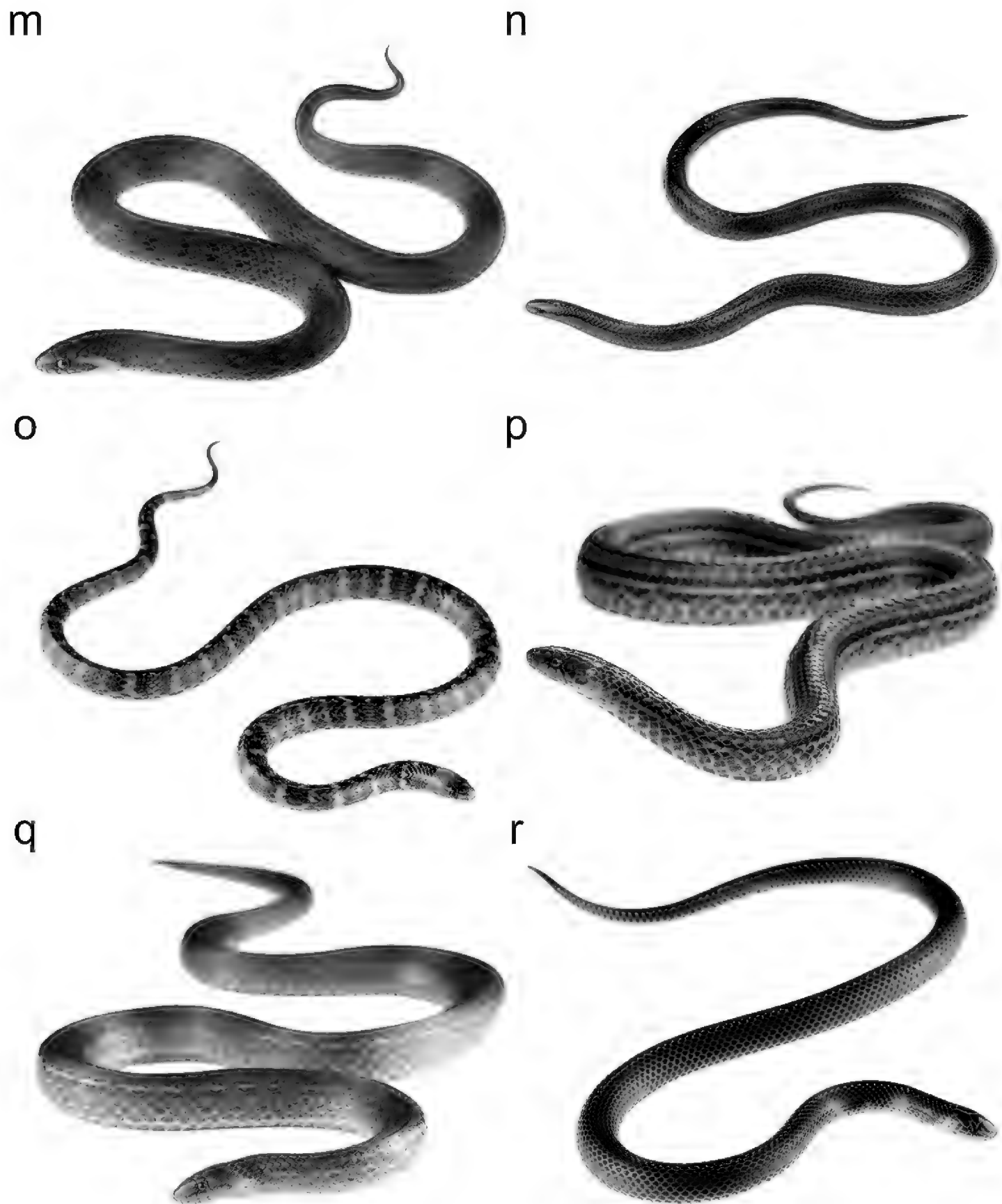


Figure 2. Continued.

**Table 3.** Morphometric data for members of the *Atractus roulei* species group. Codes are: V=ventrals; SC=subcaudals; D=dorsal scale rows at midbody; PO=postoculars; SL=supralabials; IL=infralabials; MT=maxillary teeth. Data is derived from Appendix III and from the literature.

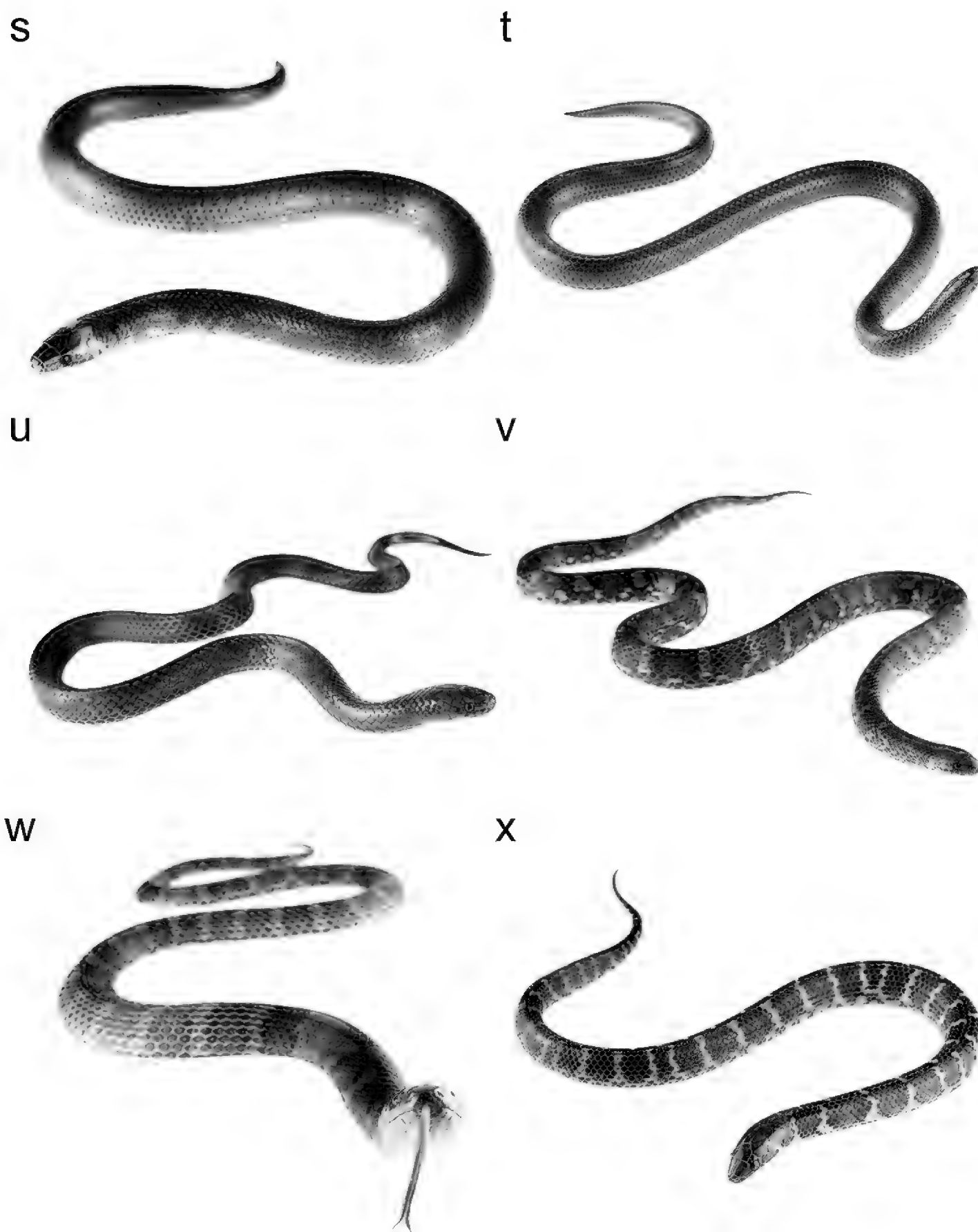
Species	V		SC		D	PO	SL	IL	MT
	Males	Females	Males	Females					
<i>A. carrioni</i>	136–151	143–161	25–34	18–32	15	1	6	6	7–10
<i>A. lehmanni</i>	141–144	148–153	25–29	20–21	15–17	1	5	6	8–11
<i>A. pyroni</i>	–	143	–	16	15	1	6	5	8
<i>A. roulei</i>	135–146	143–156	20–27	14–23	15	1	5–6	6–7	9–13



**Figure 2.** Continued.

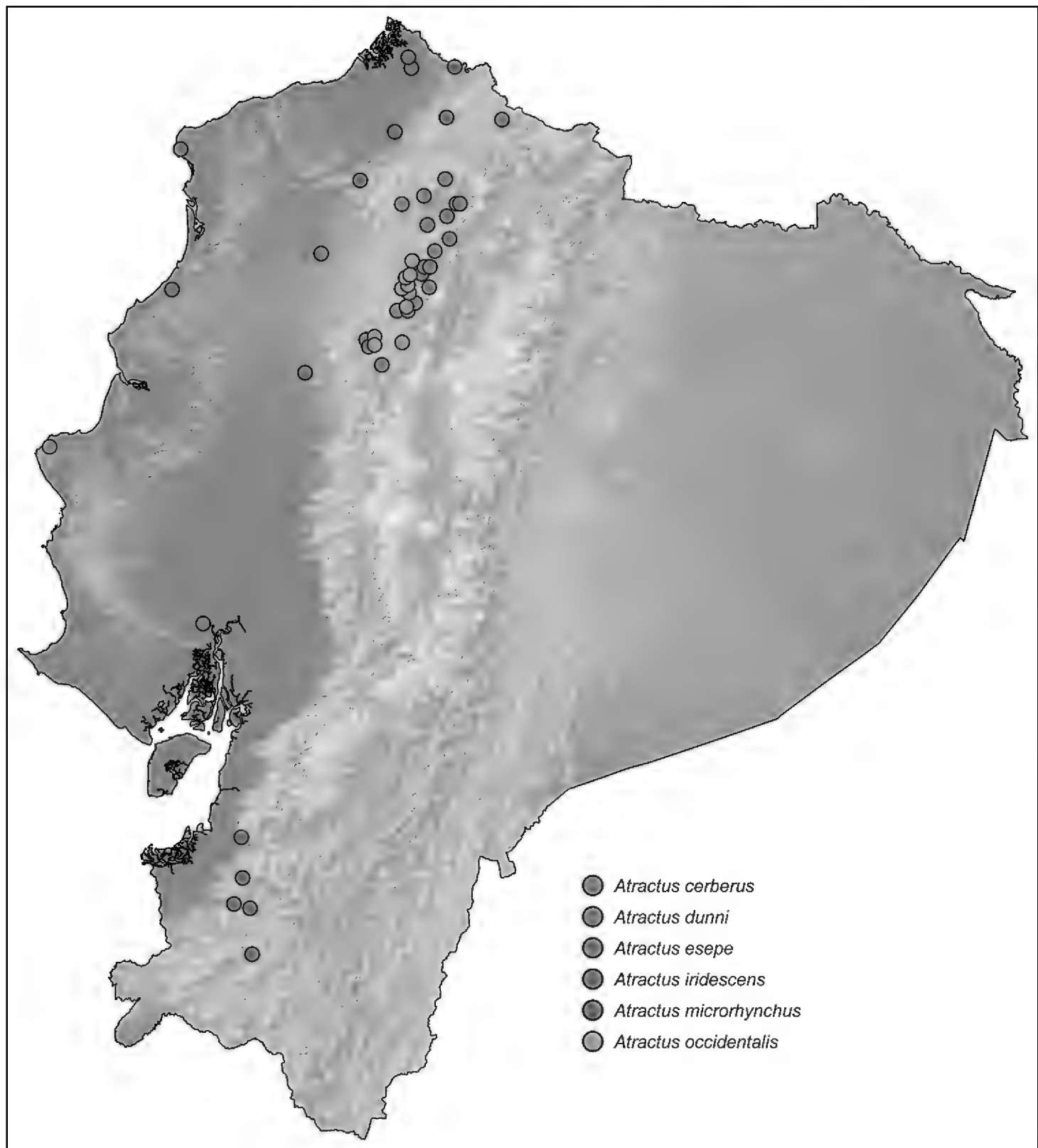
their unusual combination of 15/15/15 dorsals and 6 supralabials. Our examination of new material belonging to these two species, and material belonging to *A. pyroni* and *A. roulei* (Appendix III), shows that although the majority of specimens have indeed 6 supralabials, some specimens may have 5, compared with most Ecuadorian *Atractus* which have 7 (Appendix III). One specimen of *A. roulei* from the type locality (MZUTI 4544; Table 1) lacks a loreal scale, which was long thought (Savage 1960;





**Figure 2.** Continued.

Passos et al. 2013) to be the main feature separating this species from *A. carrioni*. The syntype of *A. lehmanni* (MC 33513) revised by Savage (1960) has 17/17/17 dorsal scale rows. Specimens assignable to *A. lehmanni* have been found only in the vicinity of the type locality (hoya de Cuenca; see Table 1).



**Figure 3.** Distribution of Ecuadorian snakes of the *Atractus iridescens* species group. Dots represent known localities.

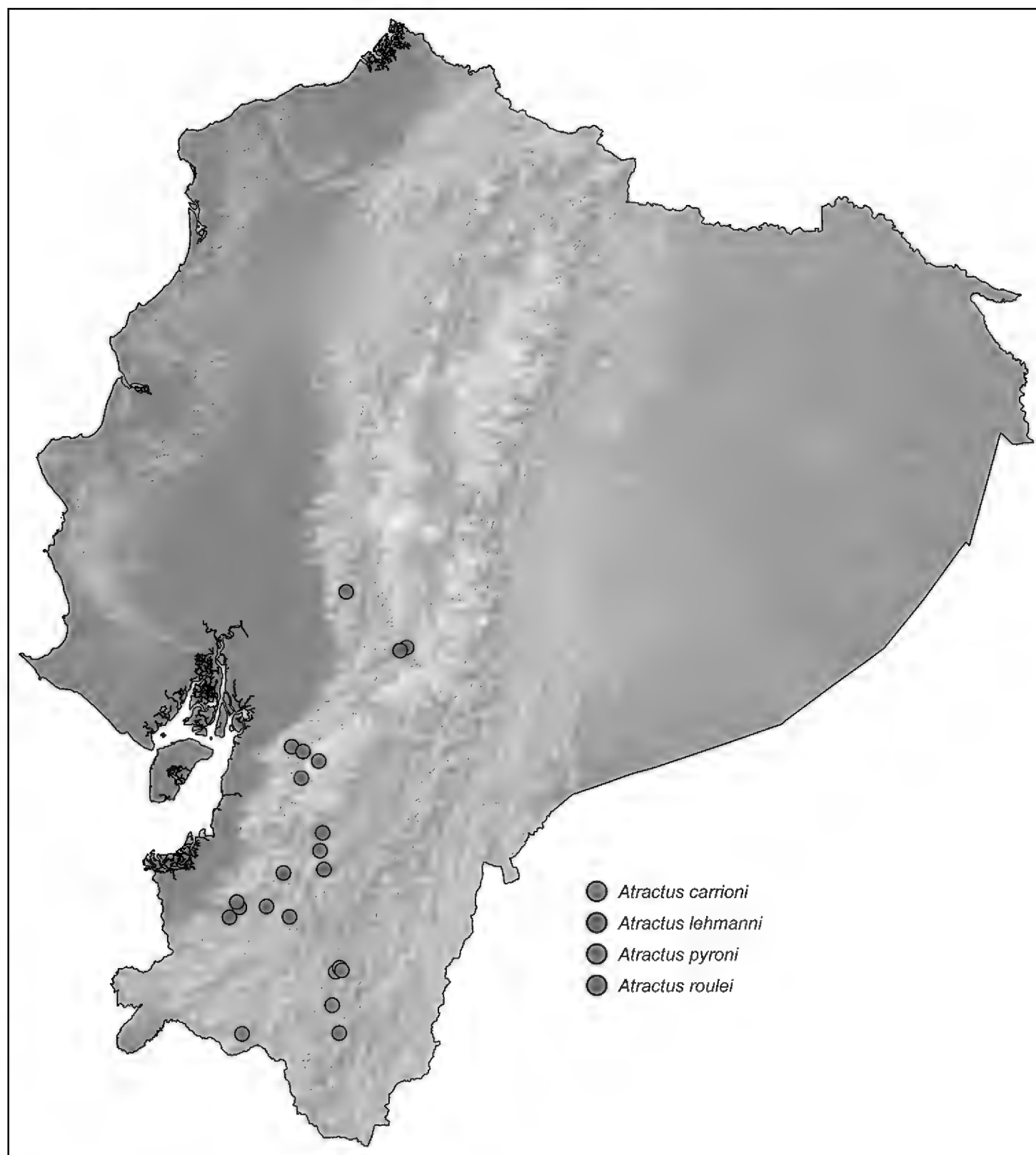
***Atractus cerberus* sp. n.**

<http://zoobank.org/B93B0063-06B6-462F-8C4B-7559D9459714>

**Proposed standard English name.** Cerberus Groundsnake

**Proposed standard Spanish name.** Tierrera cancerbera

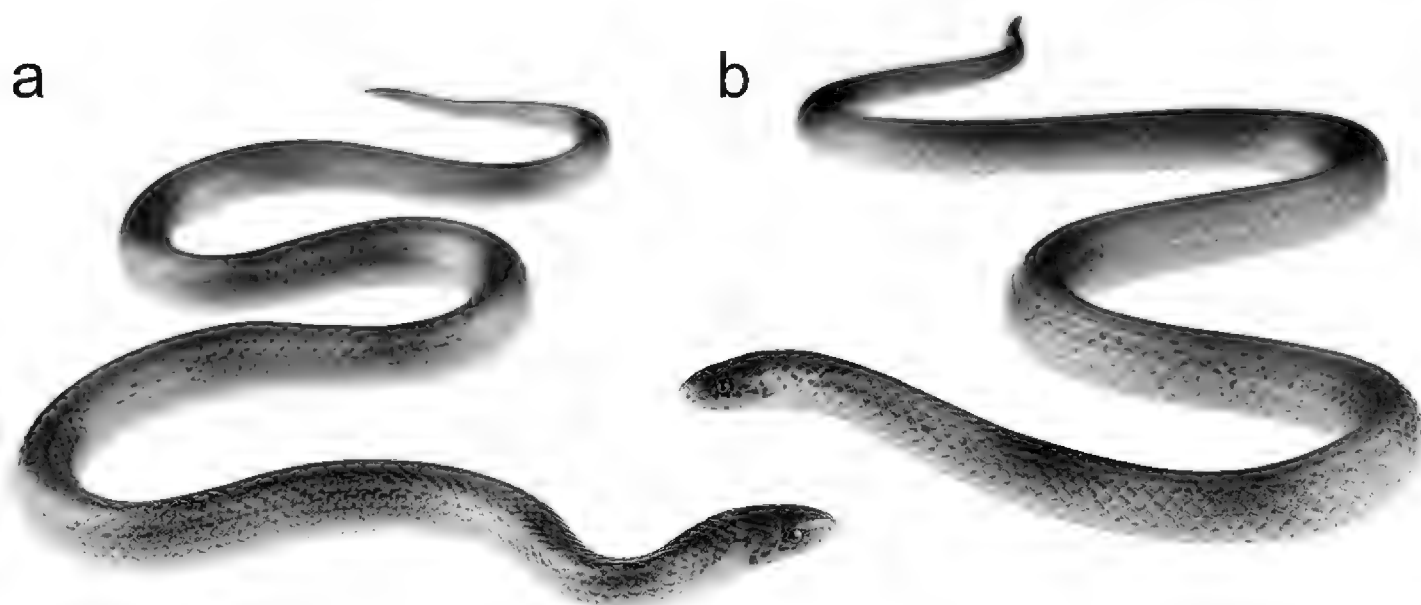
**Holotype.** MZUTI 4330 (Fig. 5a), adult male collected by José L. Vieira-Fernandes and Alejandro Arteaga on November 06, 2015 at Pacoche, province of Manabí, Ecuador (S1.06664, W80.88123; 280 m).



**Figure 4.** Distribution of Ecuadorian snakes of the *Atractus roulei* species group. Dots represent known localities.

**Paratopotype.** MZUTI 5108 (Fig. 5b), adult male collected by Alejandro Arteaga on September 04, 2016.

**Diagnosis.** *Atractus cerberus* is placed in the genus *Atractus* as diagnosed by Savage (1960), based on phylogenetic evidence (Fig. 1). It is included in the *A. iridescens* group due to its brown dorsal ground color (Fig. 5) and its phylogenetic position (Fig. 1). The species is diagnosed based on the following combination of characters: (1) 17/17/17 smooth dorsals; (2) two postoculars; (3) loreal moderate; (4) temporals 1+2; (5) seven supralabials, third and fourth contacting orbit; (6) seven infralabials, first four contacting chinshields (7) seven maxillary teeth; (8) three gular scale rows; (9)



**Figure 5.** Adult male holotype MZUTI 4330 (**a**) and adult male paratopotype (**b**) of *Atractus cerberus* MZUTI 5108.

three preventrals; (10) 152–157 ventrals; (11) 25–26 subcaudals; (12) dorsal ground color brown with faint black longitudinal bands (Fig. 5); (13) venter light yellow faintly speckled with brownish pigment; (14) 212–309 mm SVL; (15) 23–36 mm TL.

**Comparisons.** *Atractus cerberus* is included in the *A. iridescens* species group and compared to other Pacific lowland congeners that have a brownish ground color (Fig. 2): *A. boulengerii*, *A. dunni*, *A. echidna*, *A. esepe* sp. n., *A. iridescens*, *A. medusa*, *A. microrhynchus*, and *A. occidentalis*. From *A. boulengerii* and *A. medusa*, it differs in having a striped pattern as opposed to bold black blotches (Fig. 5). From all others, it differs in having yellow ventral surfaces (as opposed to cream or dingy white) and having more than 150 ventrals in males. Finally, the dorsal pattern of *A. cerberus* is less clearly marked than in the majority of the known specimens of the species included in the *A. iridescens* group. Instead of having conspicuous spots, blotches or lines, *A. cerberus* has a series of feebly visible dashes made of pigment slightly darker than the surrounding ground color.

**Color pattern.** The dorsal ground color is brown with five feebly visible dark-brown to black longitudinal lines that are not continuous throughout the length of the body but broken into spots along some sections (Fig. 5). Between the dark longitudinal lines on each side of the body, there are fields of lighter pigment that on some sections of the body correspond to lines. The head is darker than the rest of the dorsal surfaces and is marked by a dark, irregular postocular stripe that reaches the corner of the mouth (Fig. 5). The top of the supralabials is tinged with black. The ventral surfaces are yellowish cream with scattered brownish speckling that becomes more concentrated towards the tail, which is almost completely brown. The iris is carmine and the pupil is black.

**Description of holotype.** Adult male, SVL 212 mm, tail length 23 mm (10.8% SVL); body diameter 6.5 mm; head length 7.9 mm (3.7% SVL); head width 4.8 mm (2.3% SVL); interocular distance 3.1 mm; head slightly distinct from body; snout–or-

bit distance 2.8 mm; rostral 1.6 mm wide, about one time broader than high; internasals 1.0 mm wide; internasal suture sinistral relative to prefrontal suture; prefrontals 1.7 mm wide; frontal 2.3 mm wide, with a curvilinear triangle shape in dorsal view; parietals 2.1 mm wide, about twice as long as wide; nasal divided; loreal 1.5 mm long, about 2 times longer than high; eye diameter 1.4 mm; pupil round; supraoculars 1.4 mm wide; two postoculars; temporals 1+2, upper posterior temporal elongate, about four times longer than high, and three times as long as first temporal; seven supralabials, 3<sup>rd</sup>–4<sup>th</sup> contacting orbit; symphyisial 1.0 mm wide, about twice as broad as long, separated from chin shields by first pair of infralabials; seven infralabials, 1<sup>st</sup>–4<sup>th</sup> contacting chin shields; anterior chin shields about three times as long as broad, posterior chin shields absent; three series of gular scales; dorsal scales 17/17/17 rows, smooth without apical pits; precentrals 3; ventrals 157; anal plate single; paired subcaudals 26.

**Natural history.** The two known specimens of *Atractus cerberus* were found in an isolated patch of deciduous lowland forest surrounded by dry lowland shrubland. MZUTI 4330 was found active on leaf litter at 19h29, in 80% closed canopy secondary forest far from streams. The night was warm and there was drizzle the night before. MZUTI 5108 was found crossing a forest trail close to an open area at 10h00 during a sunny morning after a rainy night.

**Distribution.** Known only from the type locality, Pacoche, in the Ecuadorian province of Manabí at 280–324 m (Fig. 3). This locality is 3 km airline distance from the shoreline.

**Etymology.** The specific epithet “*cerberus*” is derived from the name of the Greek monster Kérberos. In Greek mythology, Kérberos is a monstrous multi-headed dog that guards the gates of the underworld, preventing the dead from leaving. Here, we use this word in allusion to the type locality, at the gates of the newly formed “Refinería del Pacífico”, a massive industrial oil-processing plant that can easily be likened to the underworld.

**Conservation status.** Although *Atractus cerberus* belongs to a poorly studied genus of snakes and is known only from two specimens collected recently in a single locality, we consider this species to be Critically Endangered following B1a,b(iii) IUCN criteria because: i) its extent of occurrence is estimated to be less than 50 km<sup>2</sup> (i.e. total area of continuous semideciduous forest in the Refugio de Vida Silvestre Pacoche); ii) it has not been detected in any other locality in the province of Manabí despite numerous surveys (Almendáriz and Carr 2007, Cisneros-Heredia 2004, MECN et al. 2013); and iii) and its habitat is severely fragmented, isolated from other such habitats and declining in extent and quality due to deforestation.

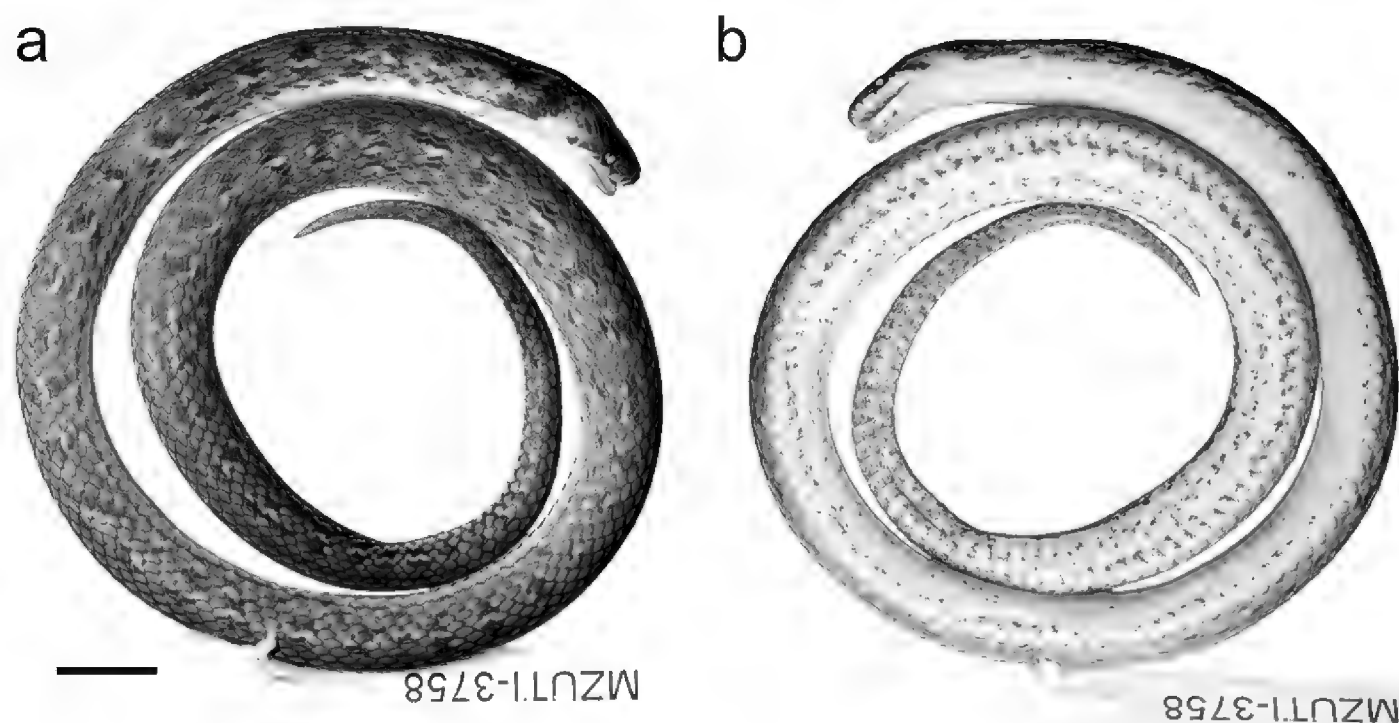
***Atractus esepe* sp. n.**

<http://zoobank.org/F58E89A5-D398-4703-8098-7474CD6B3E6D>

**Proposed standard English name.** Indistinct Groundsnake

**Proposed standard Spanish name.** Tierrera indistinta





**Figure 6.** Adult male holotype of *Atractus esepe* MZUTI 3758 in dorsal (a) and ventral (b) view. Scale = 1 cm.

**Holotype.** MZUTI 3758 (Fig. 6), adult male collected by Alejandro Arteaga on September 12, 2014 at Caimito, Esmeraldas Province, Ecuador (N0.69620, W80.090472; 102 m).

**Paratopotype.** MZUTI 3759, adult female collected by Jaime Culebras.

**Diagnosis.** *Atractus esepe* is placed in the genus *Atractus* as diagnosed by Savage (1960), based on phylogenetic evidence (Fig. 1). It is included in the *A. iridescens* group due to its brown dorsal ground color and its phylogenetic position (Figs 1, 6). The species is diagnosed based on the following combination of characters: (1) 17/17/17 smooth dorsals; (2) two postoculars; (3) loreal long; (4) temporals 1+2; (5) seven supralabials, third and fourth contacting orbit; (6) seven infralabials, first four contacting chinshields (7) seven maxillary teeth; (8) 2–3 gular scale rows; (9) 2–3 preventrals; (10) 149 ventrals in the male holotype, 156 in the female paratype; (11) 41 subcaudals in the male holotype, 30 in the female paratype; (12) dorsal ground color brown with a pattern of complete (MZUTI 3759) or broken (MZUTI 3758) (Fig. 6a) dark lines running parallel along each side of the body and separated from each other by a cream line, but rendering the appearance of a row of dorso-lateral blotches in the broken pattern (MZUTI 3758); (13) venter cream faintly speckled with brownish pigment (Fig. 6b); (14) 232–241 mm SVL; (15) 34–53 mm TL.

**Comparisons.** *Atractus esepe* is included in the *A. iridescens* species group and compared to other Pacific lowland congeners who have a brownish ground color (Figs 2, 5): *A. boulengerii*, *A. cerberus*, *A. dunni*, *A. echidna*, *A. iridescens*, *A. medusa*, *A. microrhynchus*, and *A. occidentalis*. From these, *A. microrhynchus* and *A. occidentalis* have striped pattern and cream ventral surfaces similar to that of *A. esepe*, but they occur parapatrically (Fig. 3) and can be distinguished from *A. esepe* by a genetic divergence of 5.3–5.7% in a 506 bp

long fragment of the mitochondrial Cytb gene and by having a greater number of subcaudal scales in males (Table 2). Furthermore, adult specimens of *A. microrhynchus* have light brown dorsal surfaces instead of dark brown, and their pattern can be better described as a series of blotches rather than broken longitudinal lines. Specimens of both *A. esepe* and *A. occidentalis* have a pattern of longitudinal lines, but *A. esepe* has a greater number of ventral plus caudal scales than *A. occidentalis* (more than 180 in *A. esepe*) (Table 2).

**Color pattern.** The dorsal ground color is dark brown with either six longitudinal black lines separated by lighter areas or a pattern of dark brown longitudinally arranged spots that correspond to the longitudinal lines. On each side, the line or series of dark spots along the 2<sup>nd</sup> and 3<sup>rd</sup> dorsal scale row is feebly visible, but the other lines or spots are conspicuous. The dorsal surface of the head is dark brown and there is a clearly marked dark postocular stripe running from behind the eye to the edge of the mouth (Fig. 6). The ventral surfaces are dingy white, finely speckled with brown pigment that becomes more concentrated towards the tail. The iris is carmine and the pupil is black.

**Description of holotype.** Adult male, SVL 232 mm, tail length 53 mm (22.8% SVL); body diameter 7.0 mm; head length 7.9 mm (3.4% SVL); head width 4.8 mm (2.2% SVL); interocular distance 3.4 mm; head slightly distinct from body; snout–orbit distance 3.3 mm; rostral 1.8 mm wide, about one time broader than high; internasals 0.9 mm wide; internasal suture sinistral relative to prefrontal suture; prefrontals 1.9 mm wide; frontal 2.2 mm wide, with a curvilinear triangle shape in dorsal view; parietals 2.1 mm wide, about twice as long as wide; nasal divided; loreal 2.5 mm long, about 3 times longer than high; eye diameter 1.5 mm; pupil round; supraoculars 1.2 mm wide; two postoculars; temporals 1+2, upper posterior temporal elongate, about four times longer than high, and three times as long as first temporal; seven supralabials, 3<sup>rd</sup>–4<sup>th</sup> contacting orbit; symphyseal 0.8 mm wide, separated from chin shields by first pair of infralabials; seven infralabials, 1<sup>st</sup>–4<sup>th</sup> contacting chin shields; anterior chin shields about three times as long as broad, posterior chin shields absent; three series of gular scales; dorsal scales 17/17/17 rows, smooth without apical pits; preventrals 3; ventrals 149; anal plate single; paired subcaudals 41.

**Natural history.** The two known specimens of *Atractus esepe* were found actively foraging among soil and roots in secondary evergreen lowland forest at least 400 m from the nearest natural body of water. They were found by night at 20h00 after a warm, sunny day.

**Distribution.** Known only from the type locality, Caimito, in the Ecuadorian province of Esmeraldas at 102 m (Fig. 3). This locality is 1.3 km airline distance from the shoreline.

**Etymology.** The specific epithet *esepe* is derived from the Spanish pronunciation of “sp.”, which is the abbreviation for the Latin word *species*. Here, we use this word in allusion to how the majority of Ecuadorian researchers refer to *Atractus* specimens found in the field.

**Conservation status.** We consider *Atractus esepe* to be Data Deficient following IUCN criteria because it is known only from its type locality but its occurrence in the biogeographic Choco suggests that it might as well be present in other localities. The

Chocoan forests of Caimito do not appear to be isolated from other similar habitat by geographical or ecological barriers. Therefore, we consider there is inadequate information to make a direct, or indirect, assessment of its extinction risk based on its scarce distribution data.

***Atractus pyroni* sp. n.**

<http://zoobank.org/36145E29-02B6-4C66-A097-44EFC1BC3A92>

**Proposed standard English name.** Pyron's Groundsnake

**Proposed standard Spanish name.** Tierrera de Pyron

**Holotype.** MZUTI 5107 (Fig. 7), adult male collected by José L. Vieira-Fernandes and Carlos Durán on May 23, 2016 between Balzapamba and Bilován, province of Bolívar, Ecuador (S1.83601, W79.13322; 2026 m).

**Diagnosis.** *Atractus pyroni* is placed in the genus *Atractus* as diagnosed by Savage (1960), based on phylogenetic (Fig. 1) and morphological (Table 3) evidence. It is included in the *A. roulei* group due to its 15/15/15 dorsal scale rows and its phylogenetic position (Fig. 1). The species is diagnosed based on the following combination of characters: (1) 15/15/15 smooth dorsals; (2) one postocular; (3) loreal long; (4) temporals 1+2; (5) six supralabials, third and fourth contacting orbit; (6) five infralabials, first four contacting chinshields (7) eight maxillary teeth; (8) 3 gular scale rows; (9) 2 preentrals; (10) 143 ventrals; (11) 16 subcaudals; (12) dorsal ground color dark brown with a series of light golden brown paravertebral scales running along the entire dorsum (Fig. 7); (13) venter dark brown with scattered scales of a lighter color; (14) 443 mm SVL; (15) 34 mm TL.

**Comparisons.** *Atractus pyroni* is compared to members of the *A. roulei* species group: *A. carrioni*, *A. lehmanni*, and *A. roulei* (Fig. 2). From *A. carrioni*, it differs by having a loreal. From *A. lehmanni* and *A. roulei*, it differs in size and color pattern. *Atractus pyroni* is 443 mm in SVL; whereas *A. lehmanni* is 262–321 in SVL, and *A. roulei* is 230–396. Both *A. lehmanni* and *A. roulei* have uniform dorsal ground color, whereas *A. pyroni* has a distinct dorsal bicolored pattern (Fig. 7). Finally, in life, *A. pyroni* is darker than the remaining members of the *A. roulei* species group and has a ventral pattern that, instead of having fine speckles, has conspicuous scattered blotches of a contrasting color.

**Color pattern.** The dorsal ground color is blackish with a dark vertebral (mid-dorsal) scale row flanked by a dark yellow scale row on either side (the 7<sup>th</sup> dorsal scale row), irregularly adjoined by one to few additional yellow scales on the 6<sup>th</sup> dorsal scale row, rendering an appearance of an irregularly edged mid-dorsal striped pattern (Fig. 7). The dorsal and lateral surfaces of the head are dark grayish brown and the labials are dark mustard yellow. All ventral surfaces are glossy grayish black except for the throat and some scattered blotches, which are dark mustard yellow.

**Description of holotype.** Adult female, SVL 443 mm, tail length 34 mm (7.7% SVL); body diameter 11.6 mm; head length 14.4 mm (3.3% SVL); head width 9.8 mm



**Figure 7.** Adult female holotype of *Atractus pyroni*. MZUTI 5107. Scale = 1 cm.

(2.2% SVL); interocular distance 5.1 mm; head slightly distinct from body; snout–orbit distance 5.7 mm; rostral 2.8 mm wide, about two times broader than high; internasals 1.5 mm wide; internasal suture sinistral relative to prefrontal suture; prefrontals 2.8 mm wide; frontal 3.5 mm wide, with a curvilinear triangle shape in dorsal view; parietals 4.0 mm wide, about twice as long as wide; nasal divided; loreal 3.7 mm long, about 3 times longer than high; eye diameter 1.8 mm; pupil round; supraoculars 2.1 mm wide; one postocular; temporals 1+2, upper posterior temporal elongate, about five times longer than high, and twice as long as first temporal; six supralabials, 3<sup>rd</sup>–4<sup>th</sup> contacting orbit; symphyseal 2.4 mm wide, separated from chin shields by first pair of infralabials; five infralabials, 1<sup>st</sup>–4<sup>th</sup> contacting chin shields; anterior chin shields about three times as long as broad, posterior chin shields absent; three series of gular scales; dorsal scales 15/15/15 rows, smooth without apical pits; prefrontals 2; ventrals 143; anal plate single; paired subcaudals 16.

**Natural history.** The only known specimen of *Atractus pyroni* was found dead on a dirt road surrounded by silvopastures and remnants of native montane cloudforest.

**Distribution.** Known only from the type locality, between Balzapamba and Bilován, in the Ecuadorian province of Bolívar at 2026 m (Fig. 7).

**Etymology.** Named after R. Alexander Pyron, one of the most prolific contemporary herpetologists, in recognition of his invaluable contribution to systematics and evolution of the world's reptiles.

**Conservation status.** We consider *Atractus pyroni* to be Data Deficient following IUCN because there is inadequate information to make a direct, or indirect, assessment of its extinction risk based on its scarce distribution data.

## Discussion

Species relationships and taxonomy in the colubrid snake genus *Atractus* are still far from being resolved, and many infrageneric groups are either non-monophyletic, or poorly supported and weakly placed, which may reflect inadequate sampling of taxa (only 30 out of 140 species are included) or characters (only 1 locus is used). No monophyly was found for the groups defined by Savage (1960), which, until further phylogenetic evidence is accumulated or unambiguous diagnostic characters are defined, should not be used.

From the five members of the *A. paucidens* species groups of Passos et al. (2009a) that were sampled in our phylogeny, only *A. paucidens*, *A. savagei*, and *A. typhon* cluster together. *Atractus microrhynchus* and *A. iridescens* belong to another lineage, which is here named the *A. iridescens* species group. This group includes the aforementioned two species plus *A. cerberus*, *A. dunni*, *A. echidna*, *A. esepe*, and *A. occidentalis*. From the species included in this group, we expand the known distribution of all their members (Fig. 3). However, we do not include the specimens ANSP 18114 nor ANSP 26316, from the vicinity of Huigra and identified as *A. occidentalis* by Savage (1960), because their description disagrees with the observed morphological variation reported for *A. occidentalis* in this work. Upon a visit to Huigra, a dry valley dominated by xeric vegetation and rocky outcrops, it became clear to us that it is unlikely for a species like *A. occidentalis*, which is found in evergreen lower-montane forests (Arteaga et al. 2013), to occur in an isolated dry habitat type ca. 250 km airline distance south of the type locality.

We also re-delimit the *A. roulei* species group of Passos et al. (2013) to include *A. carrioni*, *A. lehmanni*, *A. roulei* and *A. pyroni*. We expand the known distribution of *A. roulei* (Fig. 4), but do not include specimen AMNH 17492 from San José de Chimbo (Savage 1960) in the map because this specimen might actually be *A. pyroni* given the morphological similarities between the two species and the geographical proximity to the type locality of *A. pyroni*. Reports of *A. lehmanni* from Colombia (Passos et al. 2009b) are likely misidentifications since *A. lehmanni* has not been registered in Ecuador outside the type locality.

To further clarify the landscape of *Atractus* taxonomy in Ecuador, we analyze the presence of *A. medusa*, *A. melas*, *A. typhon*, *A. badius*, and *A. bocourti* in the country. Cisneros-Heredia and Romero (2015) presented the first country record of *A. medusa* in Ecuador (specimen DFCH-USFQ 191.101109 at Universidad San Francisco de Quito), based on similarities in scalation and coloration between that specimen and the holotype of *A. medusa*, from Gorgona island, Colombia. Certainly, the characters of scalation of the Ecuadorian specimen fit the diagnosis of *A. medusa*. However, they fit just as well the diagnosis of *A. iridescens* provided by Passos et al. (2009a), with the difference that the dorsal pattern of the Ecuadorian specimen resembles more the *A. iridescens* specimen, ICN 10902, pictured in Passos et al. (2009a). The dark brown ground color (as opposed to light cream), the light bordered brown blotches (as opposed to



solid black blotches), and the absence of a black nape band are all characteristics shared by DFCH-USFQ 191.101109 and the other nine specimens of *A. iridescens* presented in Appendix III, with ICN 10902 of Passos et al. (2009a). Therefore, we consider that DFCH-USFQ 191.101109 actually represents the first country record of *A. iridescens* for Ecuador. Based on this new information and re-examination of museum material, we report on 9 additional specimens (Table 1) that expand the current known distribution of this species. Cisneros-Heredia and Romero (2015) suggest that a photographic record of *Atractus* cf. *melas* from the Bilsa Biological Station, province of Esmeraldas, northwestern Ecuador (Ortega-Andrade et al. 2010) corresponds to *A. multicinctus*. The specimen differs from other material assigned to *A. multicinctus* in having whitish rings as opposed to red rings throughout the body (Fig. 2). Although photographic vouchers of *A. typhon* have been presented in MECN et al. (2013), we report on the first museum vouchers of the species in Ecuador (Table 1).

Finally, although Hoogmoed (1980) restricted the type locality of *A. badius* and pointed out that the upper Amazon basin specimens were misidentifications, the species has remained in Ecuadorian faunal lists (Torres-Carvajal et al. 2016), even after Schargel et al. (2013) made compelling cases to exclude this species from the upper Amazon Basin. Other snake, *A. bocourti* was included in the herpetofauna of Ecuador by Pérez-Santos and Moreno (1991) without pointing out to any museum voucher. These authors stated that although they have no information about the distribution of the species in Ecuador, its distribution in Colombia would suggest that it also occurs in Ecuador. Since there is no evidence that neither *A. badius* nor *A. bocourti* occur in Ecuador, we remove them from this country's herpetofauna.

Our analysis of new *Atractus* material supports the evolutionary phylogenetic distinctiveness of at least 22 of the total taxa currently recognized to occur in Ecuador. To include the remaining taxa in future phylogenetic analyses will certainly help resolve species relationships and taxonomic arrangements of cis-Andean Ecuadorian *Atractus*, since the five species that were not included in the phylogeny occur in the Amazonian slopes of the Andes. However, besides including more taxa in future phylogenetic analyses, we feel that a more adequate sampling of molecular markers is needed to overcome the difficulties that mitochondrial-based phylogenies have to capture higher-level evolutionary relationships. Certainly, future studies can benefit from a phylogeny based on both a nuclear and a mitochondrial dataset.

With these changes, the species number reported in Ecuador increases to 27: *A. carrioni* (Parker, 1930), *A. cerberus*, *A. collaris* (Peracca, 1897), *A. duboisi* (Boulenger, 1880), *A. dunni* (Savage, 1955), *A. ecuadorensis* (Savage, 1955), *A. elaps* (Günther, 1858), *A. esepe*, *A. gaigeae* (Savage, 1955), *A. gigas* (Myers and Schargel, 2006), *A. iridescens* (Peracca, 1860), *A. lehmanni* (Boettger, 1898), *A. major* (Boulenger, 1894), *A. microrhynchus* (Cope, 1868), *A. modestus* (Boulenger, 1894), *A. multicinctus* (Jan, 1865), *A. occidentalis* (Savage, 1955), *A. occipitoalbus* (Jan, 1862), *A. orcesi* (Savage, 1955), *A. paucidens* (Despax, 1910), *A. pyroni*, *A. resplendens* (Werner, 1901), *A. roulei* (Despax, 1910), *A. savage* (Salazar-Valenzuela et al., 2014), *A. snethlageae* (da Cunha & do Nascimento, 1983), *A. touzeti* (Schargel et al., 2013) and *A. typhon* (Passos et al., 2009).

We hope that the novel genetic and morphological data provided herein will promote future researchers to examine species boundaries in *Atractus*, as additional work clearly is waiting.

### Author contributions

Conceived and designed the work: AA. Performed the analyses: AA NP. Gathered morphological data: KB JHV DFCH CRP JLVF AA. Analyzed the data: AA KM DFCH JMG. Contributed reagents/materials/analysis tools: JMG NP. Wrote the paper: AA KM JHV DFCH NP CRP JLVF JMG.

### Acknowledgments

This article was greatly improved by comments of three anonymous reviewers. For granting access to their protected forests, we are grateful to Francisco Sornoza and Martin Schaefer of Fundación Jocotoco, Ana Cristina de la Torre of Pacoche Lodge, and Andrés Chiriboga of Tundaloma Lodge. Special thanks to Lucas Bustamante, Alex Pyron, Gabriela Morales, Carlos Durán, Carlos Gómez, Gabriela Aguiar, James Muchmore, Ryan Lynch, Rita Hidalgo, Ángela León, Silvia Cevallos, Paulina Romero, Jaime Culebras, Carlos Londoño, Andy Proaño, and Daniel Romero for their assistance and companionship in the field. For providing the picture of *Atractus iridescens* QCAZ 8072, we are grateful to Luis A. Coloma. For granting access to specimens under their care, we are grateful to Mario H. Yáñez-Muñoz (DHMECN), Ana Almendáriz (EPN), Katty Garzón-Tello and Maria Helena Barragán (FHGO), Andreas Schmitz (MHNG), Nicolas Vidal (MNHN), David Salazar-Valenzuela (MZUTI), Omar Torres-Carvajal (QCAZ), Kevin de Queiroz (USNM), and Juan Carlos Sánchez-Nivicela (UDA). Fieldwork was made possible with the support of Tropical Herping and Universidad Tecnológica Indoamérica. Laboratory work was carried out at Universidad Tecnológica Indoamérica in Quito. Sequencing was made possible with support of the George Washington University, Universidad Tecnológica Indoamérica, and the U.S. National Science Foundation.

### References

- Almendáriz A, Carr JL (2007) Lista actualizada de los anfibios y reptiles registrados en los remanentes de bosque de la Cordillera de la Costa y áreas adyacentes del suroeste de Ecuador. Escuela Politécnica Nacional, Quito, 11 pp.
- Arévalo E, Davis SK, Sites JW (1994) Mitochondrial DNA-sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. *Systematic Biology* 43: 387–418. <https://doi.org/10.1093/sysbio/43.3.387>

- Arteaga A, Bustamante L, Guayasamin JM (2013) The amphibians and reptiles of Mindo. Universidad Tecnológica Indoamérica, Quito, 257 pp.
- Boettger O (1898) Katalog der Reptilien-Sammlung im Museum der Senckenbergischen Naturforschenden Gesellschaft in Frankfurt. II Teil (Schlangen). Gebrüder Knauer, Frankfurt, 160 pp.
- Boulenger GA (1880) Reptiles et Batraciens recueillis par M. Emile de Ville dans les Andes de l'équateur. Bulletin de la Société Zoologique de France 5: 41–48.
- Boulenger GA (1894) Catalogue of the snakes in the British Museum (Natural History), Volume II. Trustees of the British Museum, London, 382 pp.
- Burbrink FT, Lawson R, Slowinski JB (2000) Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): A critique of the subspecies concept. Evolution 54: 2107–2188. <https://doi.org/10.1111/j.0014-3820.2000.tb01253.x>
- Campbell HW, Christman SP (1982) Field techniques for herpetofaunal community analysis. In: Scott NJ (Ed.) Herpetological communities: a symposium of the Society for the Study of Amphibians and Reptiles and the Herpetologists League. United States Fish and Wildlife Service, Washington D.C., 193–200.
- Cisneros-Heredia DF (2004) Amphibians, Machalilla National Park, province of Manabí, western Ecuador. Check List 2: 45–54. <https://doi.org/10.15560/2.1.45>
- Cisneros-Heredia DF (2005) Rediscovery of the Ecuadorian snake *Atractus dunni* Savage, 1955 (Serpentes: Colubridae). Journal of the National Museum (Prague), Natural History Series 174: 87–94.
- Cisneros-Heredia DF, Romero A (2015) First country record of *Atractus medusa* (Serpentes, Dipsadidae) in Ecuador. Herpetology Notes 8: 417–420.
- Cope ED (1868) An examination of the Reptilia and Batrachia obtained by the Orton Expedition to Ecuador and the Upper Amazon, with notes on other species. Proceedings of the Academy of Natural Sciences of Philadelphia 20: 96–140.
- da Cunha OR, do Nascimento FP (1983) Ofidios da Amazonia. As especies de *Atractus* Wagler, 1828, na Amazonia oriental & Maranhao (Ophidia, Colubridae). Boletim do Museu Paraense Emilio Goeldi 123: 1–38.
- Darriba D, Taboada GL, Doallo R, Posada D (2012) More models, new heuristics and parallel computing. Nature Methods 9: 772–772. <https://doi.org/10.1038/nmeth.2109>
- De Oliveira EA, Hernández-Ruz EJ (2016) Morphological variation in *Atractus tartarus* (Snake: Dipsadidae) from the Xingu River, east Amazon, Brazil and preliminary phylogenetic relationship in *Atractus*. International Journal of Research Studies in Biosciences 4: 1–7.
- Despax R (1910) Mission géodésique de l'Équateur. Collections recueillies par M. le Dr Rivet. Liste des ophidiens et descriptions des espèces nouvelles. Bulletin du Muséum National d'Histoire Naturelle 16: 368–376.
- Dowling HG (1951) A proposed standard system of counting ventrals in snakes. British Journal of Herpetology 1: 97–99.
- Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Heled J, Kearse M, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2010) Geneious v5.5. Biomatters. <http://www.geneious.com>

- Dunn ER, Bailey JR (1939) Snakes from the uplands of the Canal Zone and of Darien. Bulletin of the Museum of Comparative Zoology at Harvard College 86: 1–22.
- Felsenstein J (2004) Inferring Phylogenies. Sinauer Associates, Sunderland, 664 pp.
- Gasc JP, Rodrigues MT (1979) Une nouvelle espèce du genre *Atractus* (Colubridae, Serpentes) de la Guyane française. Bulletin du Muséum national d'Histoire naturelle 2: 547–557.
- Günther A (1858) Catalogue of Colubrine snakes of the British Museum. Taylor and Francis, London, 281 pp.
- Hoogmoed MS (1980) Revision of the genus *Atractus* in Surinam, with the resurrection of two species (Colubridae, Reptilia). Notes on the Herpetofauna of Surinam VII. Zoologische Verhandelingen 175: 1–47.
- Jan G (1862) Enumerazione sistematico delle specie d'ofidi del gruppo Calamaridae. Archivio per la zoologia, l'anatomia e la fisiologia 2: 1–76.
- Jan G (1865) Iconographie générale des ophidiens. 10. JB Baillière et fils, Paris, 42 pp.
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. <https://doi.org/10.1093/molbev/mst010>
- Krysko KL, Steadman DW, Nuñez LP, Lee DS (2015) Molecular phylogeny of Caribbean dipsadid (Xenodontinae: Alsophiini) snakes, including identification of the first record from the Cay Sal Bank, The Bahamas. Zootaxa 4028: 441–450. <https://doi.org/10.11646/zootaxa.4028.3.9>
- Lanfear R, Calcott B, Ho SY, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29(6): 1695–1701. <https://doi.org/10.1093/molbev/mss020>
- MECN, JOCOTOCO, ECOMINGA (2013) Herpetofauna en áreas prioritarias para la conservación: El sistema de reservas Jocotoco y Ecominga. Museo Ecuatoriano de Ciencias Naturales, Quito, 408 pp.
- Myers CW, Schargel WE (2006) Morphological extremes - two new snakes of the genus *Atractus* from northwestern South America (Colubridae: Dipsadinae). American Museum Novitates 3532: 1–13. [https://doi.org/10.1206/0003-0082\(2006\)3532\[1:MENSOT\]2.0.CO;2](https://doi.org/10.1206/0003-0082(2006)3532[1:MENSOT]2.0.CO;2)
- Ortega-Andrade HM, Bermingham J, Aulestia C, Paucar C (2010) Herpetofauna of the Bilsa Biological Station, province of Esmeraldas, Ecuador. Check List 6: 119–154. <https://doi.org/10.15560/6.1.119>
- Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991) The simple fool's guide to PCR, version 2.0. University of Hawaii, Honolulu, 94 pp.
- Parker HW (1930) A new colubrine snake from Ecuador. The Annals and Magazine of Natural History 10: 207–209. <https://doi.org/10.1080/00222933008673120>
- Passos P, Arredondo JC, Fernandes R, Lynch JD (2009b) Three new *Atractus* (Serpentes: Dipsadidae) from the Andes of Colombia. Copeia 2009: 425–436. <https://doi.org/10.1643/CH-08-063>
- Passos P, Cisneros-Heredia D, Rivera DE, Aguilar C, Schargel WE (2012) Rediscovery of *Atractus microrhynchus* and reappraisal of the taxonomic status of *A. emersoni* and *A. natans* (Serpentes: Dipsadidae). Herpetologica 68: 375–392. <https://doi.org/10.1655/HERPETOLOGICA-D-11-00078.1>

- Passos P, Echevarría LY, Venegas PJ (2013) Morphological Variation of *Atractus carrioni* (Serpentes: Dipsadidae). South American Journal of Herpetology 2: 109–120. <https://doi.org/10.2994/SAJH-D-12-00025.1>
- Passos P, Lynch JD (2010) Revision of *Atractus* (Serpentes: Dipsadidae) from Middle and Upper Magdalena Drainage of Colombia. Herpetological Monographs 24: 149–173. <https://doi.org/10.1655/09-041.1>
- Passos P, Mueses-Cisneros JJ, Lynch JD, Fernandes R (2009a) Pacific lowland snakes of the genus *Atractus* (Serpentes: Dipsadidae), with description of three new species. Zootaxa 2293: 1–34.
- Passos P, Prudente ALC, Lynch JD (2016) Redescription of *Atractus punctiventris* and description of two new *Atractus* (Serpentes: Dipsadidae) from Brazilian Amazonia. Herpetological Monographs 30: 1–20. <https://doi.org/10.1655/HERPMONOGRAPHS-D-14-00009>
- Patwardhan A, Ray S, Roy A (2014) Molecular markers in phylogenetic studies-A review. J Phylogen Evolution Biol 2: 1–9.
- Peracca MG (1896) Sopra alcuni Ofidii nuovi o poco noti dell’America meridionale. Bollettino dei Musei di Zoologia e Anatomia Comparata della R. Università di Torino 11: 1–4.
- Peracca MG (1897) Intorna ad una piccola raccolta di Rettili di Cononacco (Perù orientale). Bollettino dei Musei di Zoologia e Anatomia Comparata della R. Università di Torino 12: 1–7.
- Pérez-Santos C and Moreno AG (1991) Serpientes de Ecuador. Museo Regionale di Scienze Naturali, Torino, 538 pp.
- Pyron RA, Guayasamin JM, Peñafiel N, Bustamante L, Arteaga A (2015) Systematics of Nothopsini (Serpentes, Dipsadidae), with a new species of *Synophis* from the Pacific Andean slopes of southwestern Ecuador. ZooKeys 541: 109–147. <https://doi.org/10.3897/zookeys.541.6058>
- Pyron RA, Arteaga A, Echevarría LY, Torres-Carvala O (2016) A revision and key for the tribe Diaphorolepidini (Serpentes: Dipsadidae) and checklist for the genus *Synophis*. Zootaxa 4171: 293–320. <https://doi.org/10.11646/zootaxa.4171.2.4>
- Rambaut A, Drummond AJ (2003) Tracer version 1.4.1. <http://beast.bio.ed.ac.uk>
- Ronquist F, Huelsenbeck JP (2013) MrBayes 3: Bayesian phylogenetic inference under mixed-models. Bioinformatics 19: 1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Salazar-Valenzuela D, Torres-Carvajal O, Passos P (2014) A New Species of *Atractus* (Serpentes: Dipsadidae) from the Andes of Ecuador. Herpetologica 70: 350–363. <https://doi.org/10.1655/HERPETOLOGICA-D-13-00045>
- Savage JM (1955) Descriptions of new colubrid snakes, genus *Atractus*, from Ecuador. Proceedings of the Biological Society of Washington 68: 11–20.
- Savage JM (1960) A revision of the Ecuadorian snakes of the colubrid genus *Atractus*. Miscellaneous Publications, Museum of Zoology, University of Michigan 112: 1–86.
- Schargel WE, Lamar WW, Passos P, Valencia JH, Cisneros-Heredia DF, Campbell JA (2013) A new giant *Atractus* (Serpentes: Dipsadidae) from Ecuador, with notes on some other large Amazonian congeners. Zootaxa 3721: 455–474. <https://doi.org/10.11646/zootaxa.3721.5.2>
- Swofford DL (2002) PAUP\*: Phylogenetic analysis using parsimony (\*and other methods). v.4.0b10. Sinauer & Associates, Sunderland, Massachusetts. <https://doi.org/10.1111/j.0014-3820.2002.tb00191.x>



- Torres-Carvajal O, Salazar-Valenzuela D, Merino-Viteri A, Nicolalde DA (2016) ReptiliaWebEcuador. Versión 2016.0. <http://zoologia.puce.edu.ec> [accessed 12 Nov 2016]
- Uetz P, Freed P, Hošek J (2016) The Reptile Database. <http://www.reptile-database.org> [accessed 7 Oct 2016]
- Werner F (1901) Über Reptilien und Batrachier aus Ecuador und Neu-Guinea. Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien 51: 593–614. <https://doi.org/10.5962/bhl.part.4586>

## Appendix I

GenBank accession numbers for loci and terminals of taxa and outgroups sampled in this study. Novel sequence data produced in this study are marked with an asterisk (\*).

Species	Voucher	16S	CYTB	ND4
<i>A. albuquerquei</i>	–	GQ457726	JQ598918	–
<i>A. badius</i>	–	AF158485	–	–
<i>A. carrioni</i>	MZUTI 4195	KY610046*	–	KY610094*
<i>A. cerberus</i>	MZUTI 4330	KY610047*	KY610073*	KY610095*
<i>A. duboisi</i>	MZUTI 62	KT944041	–	KT944059
<i>A. dunni</i>	MZUTI 2189	KY610048*	–	KY610096*
<i>A. dunni</i>	MZUTI 3031	KY610049*	–	KY610097*
<i>A. dunni</i>	MZUTI 4318	KY610050*	KY610074*	KY610098*
<i>A. dunni</i>	MZUTI 4319	KY610051*	KY610075*	KY610099*
<i>A. ecuadorensis</i>	DHMECN 5105	–	–	KY610100*
<i>A. elaps</i>	DHMECN 10179	KY610052*	KY610076*	KY610101*
<i>A. elaps</i>	KU 214837	–	EF078536	EF078584
<i>A. esepe</i>	MZUTI 3758	KY610053*	KT944052	KY610102*
<i>A. esepe</i>	MZUTI 3759	KT944039	KT944051	KT944058
<i>A. flammigerus</i>	MNHN 1997.2145	AF158471	–	–
<i>A. gigas</i>	MZUTI 3286	KT944043	KT944053	KT944061
<i>A. iridescens</i>	DHMECN 9633	KY610054*	KY610077*	–
<i>A. iridescens</i>	MZUTI 3548	KY610055*	KY610078*	–
<i>A. iridescens</i>	MZUTI 3680	KY610056*	KY610079*	–
<i>A. iridescens</i>	MZUTI 4178	KT944040	KY610080*	–
<i>A. iridescens</i>	MZUTI 4697	KY610057*	KY610081*	–
<i>A. lehmanni</i>	DHMECN 7644	KY610058*	KY610082*	KY610103*
<i>A. major</i>	ANF 1545	KT944045	–	KY610104*
<i>A. major</i>	DHMECN 8343	KY610059*	–	KY610105*
<i>A. microrhynchus</i>	MZUTI 5109	KY610060*	KY610083*	KY610106*
<i>A. microrhynchus</i>	MZUTI 4122	KT944037	KT944049	KT944056
<i>A. modestus</i>	MZUTI 4760	KY610061*	KY610084*	KY610107*
<i>A. multicinctus</i>	MZUTI 5106	KY610062*	KY610085*	KY610108*

Species	Voucher	16S	CYTB	ND4
<i>A. occidentalis</i>	MZUTI 1385	KY610063*	KY610086*	KY610109*
<i>A. occidentalis</i>	MZUTI 2649	KY610064*	KY610087*	KY610110*
<i>A. occidentalis</i>	MZUTI 2650	KT944038	KT944050	KT944057
<i>A. occidentalis</i>	MZUTI 3323	KY610065*	KY610088*	KY610111*
<i>A. paucidens</i>	MZUTI 5102	KY610066*	–	KY610112*
<i>A. paucidens</i>	MZUTI 5104	–	–	KY610113*
<i>A. paucidens</i>	MZUTI 5105	KY610067*	–	KY610114*
<i>A. pyroni</i>	MZUTI 5107	KY610068*	KY610089*	KY610115*
<i>A. resplendens</i>	MZUTI 3996	KT944042	KT944055	KT944060
<i>A. roulei</i>	MZUTI 4503	–	KY610090*	KY610116*
<i>A. roulei</i>	MZUTI 4544	KY610069*	KY610091*	KY610117*
<i>A. savagei</i>	MZUTI 4916	KY610070*	KY610092*	KY610118*
<i>A. schach</i>	–	AF158486	–	–
<i>A. touzeti</i>	ANF 2390	KY610071*	KY610093*	KY610119*
<i>A. trihedrurus</i>	–	GQ457727	JQ598919	–
<i>A. typhon</i>	DHMECN 9632	KY610072*	–	KY610120*
<i>A. typhon</i>	MZUTI 3284	KT944044	KT944054	KT944062
<i>A. wagleri</i>	MHUA 14368	–	GQ334480	GQ334581
<i>A. zebrinus</i>	–	JQ598861	–	–
<i>A. zidocki</i>	MNHN 1997.2046	AF158487	–	–
Outgroups				
<i>Geophis godmani</i>	–	JQ598877	JQ598932	–
<i>Sibon nebulatus</i>	MVZ 233298	EU728583	EU728583	EU728583

Appendix II

List of PCR and sequencing primers and their respective PCR conditions (denaturation, annealing, extension and number of corresponding cycles) used in this study. All PCR protocols included an initial 3-min step at 94 °C and a final extension of 10 min at 72 °C.

Locus	Primer name	Sequence (5'-3')	Reference	PCR profile:
16S	16Sar-L	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)	94 °C (45 sec), 53 or 56 °C (45 sec), 72 °C (1 min) [x25-30]
	16Sbr-H-R	CCGGTCTGAACTCAGATCACGT		
Cytb	L14910	GACCTGTGATMTGAAAACCA YCGTTGT	Burbrink et al. (2000)	94 °C (1 min), 58 °C (1 min), 72 °C (2 min) [x30-36]
	H16064	CTTTGGTTTACAAGAACAATGCTTTA		
ND4	ND4	CACCTATGACTACCAAAAGCTCATGTAGAAGC	Arévalo et al. (1994)	94 °C (25 sec), 58 or 60 °C (1 min), 72 °C (2 min) [x25-30]
	Leu	CATTACTTTTACTTGGATTTGACCA		

### Appendix III

Morphometric data and sex for specimens of *Atractus* species examined. Codes are: V=ventrals; SC=subcaudals; D1–3=dorsal scale rows at neck, midbody, and vent; PO=postoculars; SL=supralabials; IL=infralabials; MT=maxillary teeth; SVL=snout-vent length (mm); TL=tail length (mm); M=Male, F=Female.

Species	Voucher	V	SC	D1	D2	D3	PO	SL	IL	MT	SVL	TL	Sex
<i>A. carrioni</i>	DHMECN 4697	144	32	15	15	15	1	6	6	7	361	59	F
<i>A. carrioni</i>	DHMECN 76	157	23	15	15	15	1	6	6	8	333	39	F
<i>A. carrioni</i>	DHMECN 7668	149	28	15	15	15	1	6	6	7	354	58	M
<i>A. carrioni</i>	MZUTI 4195	144	31	15	15	15	1	6	6	8	371	53	M
<i>A. cerberus</i>	MZUTI 5108	152	25	17	17	17	2	7	7	7	309	36	M
<i>A. cerberus</i>	MZUTI 4330	157	26	17	17	17	2	7	7	7	212	23	M
<i>A. duboisi</i>	MHNG 2457.093	166	22	15	15	15	2	7	6	6	455	34	F
<i>A. duboisi</i>	MNHN 0.6147	164	17	15	15	15	2	8	7	–	131	11	F
<i>A. dunni</i>	DHMECN 12769	141	36	17	17	17	2	6	7	7	279	39	–
<i>A. dunni</i>	DHMECN 2215	144	24	17	17	17	2	7	7	6	278	35	F
<i>A. dunni</i>	DHMECN 3527	141	24	17	17	17	2	6	6	6	352	48	F
<i>A. dunni</i>	DHMECN 3900	143	21	17	17	17	2	6	6	–	101	19	–
<i>A. dunni</i>	DHMECN 4159	129	35	17	17	17	2	5	6	6	266	65	–
<i>A. dunni</i>	EPN 3127	–	–	–	–	–	–	–	–	–	355	46	F
<i>A. dunni</i>	EPN 3128	–	–	–	–	–	–	–	–	–	295	63	M
<i>A. dunni</i>	FHGO 375	128	36	17	17	17	2	7	7	6	219	48	M
<i>A. dunni</i>	FHGO 376	143	26	17	17	17	2	7	7	5	278	33	F
<i>A. dunni</i>	FHGO 379	132	35	17	17	17	2	7	7	6	297	61	M
<i>A. dunni</i>	FHGO 91	125	35	17	17	17	2	7	7	6	231	52	M
<i>A. dunni</i>	MHNG 2441.043	145	20	17	17	17	2	7	7	6	205	22	F
<i>A. dunni</i>	MHNG 2457.091	129	34	17	17	17	2	7	6	5	197	39	M
<i>A. dunni</i>	MHNG 2464.03	136	39	16	17	17	2	7	6	5	114	22	M
<i>A. dunni</i>	MZUTI 2189	134	29	17	17	17	2	7	7	6	189	28	M
<i>A. dunni</i>	MZUTI 3031	139	24	17	17	17	2	7	7	5	329	36	F
<i>A. dunni</i>	MZUTI 4097	149	21	17	17	17	2	7	7	6	152	17	–
<i>A. dunni</i>	MZUTI 4098	130	37	17	17	17	2	7	7	6	126	19	–
<i>A. dunni</i>	MZUTI 4099	140	25	17	17	17	2	7	7	–	118	15	F
<i>A. dunni</i>	MZUTI 4100	138	24	17	17	17	2	7	7	–	335	36	F
<i>A. dunni</i>	MZUTI 4318	136	34	17	18	17	2	7	7	6	242	53	M
<i>A. dunni</i>	MZUTI 4319	129	35	15	17	17	2	7	7	5	242	53	M
<i>A. esepe</i>	MZUTI 3758	149	41	17	17	17	2	7	7	5	232	53	M
<i>A. esepe</i>	MZUTI 3759	156	30	17	17	17	2	7	7	5	241	34	F
<i>A. gaigeae</i>	MHNG 2397.044	136	34	17	17	17	2	7	7	5	129	23	M
<i>A. gigas</i>	MHNG 2250.035	168	34	19	17	17	2	6	6	3	272	40	F
<i>A. gigas</i>	MHNG 2441.02	177	31	17	17	17	2	6	6	5	1060	116	F
<i>A. iridescens</i>	DHMECN 2932	138	28	17	17	17	2	6	7	6	252	36	F
<i>A. iridescens</i>	DHMECN 5663	141	32	17	17	17	2	6	6	6	272	46	F
<i>A. iridescens</i>	DHMECN 9633	129	42	16	17	17	2	6	6	6	219	62	M
<i>A. iridescens</i>	FHGO 10443	139	32	17	17	17	2	7	7	5	204	32	F
<i>A. iridescens</i>	MZUTI 3548	131	34	17	17	17	2	7	7	6	200	44	M

Species	Voucher	V	SC	D1	D2	D3	PO	SL	IL	MT	SVL	TL	Sex
<i>A. iridescens</i>	MZUTI 3680	140	40	17	17	17	2	7	7	6	210	46	M
<i>A. iridescens</i>	MZUTI 4178	148		17	17	17	2	7		5	211	37	M
<i>A. iridescens</i>	MZUTI 4697	127	38	17	17	17	2	7	7	5	209	46	M
<i>A. lehmanni</i>	DHMECN 7644	144	29	15	15	15	1	5	6	11	300	35	M
<i>A. lehmanni</i>	DHMECN 7645	144	23	15	15	15	1	5	7	10	321	42	–
<i>A. major</i>	MNHN 0.6149	174	35	17	17	17	2	7	7	6	586	86	F
<i>A. microrhynchus</i>	DHMECN 2586	144	39	17	17	17	1	7	6	6	239	45	M
<i>A. microrhynchus</i>	FHGO 897	149	37	17	17	17	2	7	7	7	239	51	M
<i>A. microrhynchus</i>	MHNG 2307.017	133	34	18	17	17	2	7	6	5	269	55	M
<i>A. microrhynchus</i>	MHNG 2397.019	144	25	17	17	17	2	7	7	6	300	–	F
<i>A. microrhynchus</i>	MHNG 2397.02	147	26	17	17	17	2	7	6	5	225	28	F
<i>A. microrhynchus</i>	MHNG 2397.021	144	24	17	17	17	2	7	6	5	217	28	F
<i>A. microrhynchus</i>	MHNG 2459.052	137	36	17	17	17	2	7	6	5	239	53	M
<i>A. microrhynchus</i>	MZUTI 4122	163	29	17	17	17	2	7	7	7	222	27	F
<i>A. microrhynchus</i>	QCAZ 1219	147	40	17	17	17	2	7	7	7	178	37	M
<i>A. microrhynchus</i>	USNM 285473	152	26	17	17	17	2	7	7	–	335	45	F
<i>A. microrhynchus</i>	USNM 285474	163	28	17	17	17	2	7	7	–	212	21	F
<i>A. modestus</i>	DHMECN 3859		45	17	17	17	2	7	6	–	344	41	–
<i>A. modestus</i>	FHGO 2936	165	41	17	17	17	2	7	7	5	110	20	M
<i>A. modestus</i>	FHGO 44	186	27	17	17	17	2	7	7	6	294	38	F
<i>A. modestus</i>	MHNG 2397.041	146	21	15	15	15	2	7	6	6	200	23	M
<i>A. modestus</i>	MZUTI 4760	147	42	17	17	17	2	6	7	5	273	59	M
<i>A. occidentalis</i>	FHGO 385	128	37	17	17	17	2	7	7	7	188	40	F
<i>A. occidentalis</i>	MHNG 2252.079	145	20	17	17	17	2	6	7	5	262	25	F
<i>A. occidentalis</i>	MHNG 2307.068	141	35	17	17	17	2	6	7	5	272	55	M
<i>A. occidentalis</i>	MHNG 2397.028	137	38	17	17	17	2	6	7	5	117	21	M
<i>A. occidentalis</i>	MHNG 2411.085	138	35	17	17	17	2	7	7	5	253	55	M
<i>A. occidentalis</i>	MHNG 2411.086	129	33	17	17	17	2	7	6	5	122	23	M
<i>A. occidentalis</i>	MHNG 2441.044	134	37	17	17	17	2	7	7	–	274	68	M
<i>A. occidentalis</i>	MZUTI 2649	134	36	17	17	16	2	7	7	6	223	35	F
<i>A. occidentalis</i>	MZUTI 2650	149	24	17	17	17	2	7	7	–	191	21	F
<i>A. occidentalis</i>	MZUTI 3323	134	39	17	17	17	2	7	7	7	332	67	M
<i>A. paucidens</i>	DHMECN 11980	171	43	17	17	17	2	7	7	7	290	50	M
<i>A. paucidens</i>	DHMECN 3975	163	43	17	17	17	2	–	7	7	249	50	M
<i>A. paucidens</i>	EPN 8730	–	–	–	–	–	–	–	–	–	246	53	M
<i>A. paucidens</i>	EPN 8731	–	–	–	–	–	–	–	–	–	237	51	M
<i>A. paucidens</i>	MHNG 2309.065	156	46	15	15	15	2	7	6	6	196	45	M
<i>A. paucidens</i>	MNHN 1906.245	186	40	17	17	17	2	7	7	–	262	42	M
<i>A. pyroni</i>	MZUTI 5107	143	16	15	15	15	1	6	5	8	443	34	F
<i>A. roulei</i>	QCAZ 6256	135	27	15	15	15	1	6	6	9	337	48	M
<i>A. roulei</i>	QCAZ 7887	146	25	15	15	15	1	5	6	9	309	39	M
<i>A. roulei</i>	QCAZ 7902	156	19	15	15	15	1	6	7	11	392	37	F
<i>A. roulei</i>	QCAZ 9643	149	17	15	15	15	1	6	6	11	139	13	F
<i>A. roulei</i>	QCAZ 9652	143	19	15	15	15	1	6	6	13	230	21	F
<i>A. savagei</i>	DHMECN 3800	166	25	17	17	17	2	6	7	7	214	23	F
<i>A. snethlageae</i>	MNHN 1906.244	151	29	17	17	17	2	7	7	7	283	35	F

Species	Voucher	V	SC	D1	D2	D3	PO	SL	IL	MT	SVL	TL	Sex
<i>A. snethlageae</i>	MNHN 1994.1171	160	27	17	17	17	2	7	7	8	315	35	F
<i>A. touzeti</i>	ANF 2390	176	31	17	17	17	2	7	7	7	652	71	F
<i>A. trilineatus</i>	MNHN 1898.313	141	19	15	15	15	2	7	7	8	179	19	M
<i>A. trilineatus</i>	MNHN 1898.314	132	21	15	15	15	2	7	7	7	182	20	M
<i>A. typhon</i>	DHMECN 9632	153	47	15	15	15	2	7	6	7	187	31	M
<i>A. typhon</i>	FHGO 10438	166	41	15	15	15	2	7	7	6	370	68	M
<i>A. typhon</i>	FHGO 10439	158	48	16	16	16	2	7	7	7	349	87	F